High-throughput nuclear delivery and rapid expression of DNA via mechanical and electrical cell-membrane disruption

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Nuclear transfection of DNA into mammalian cells is challenging yet critical for many biological and medical studies. Here, by combining cell squeezing and electric-field-driven transport in a device that integrates microfluidic channels with constrictions and microelectrodes, we demonstrate nuclear delivery of plasmid DNA within 1 h after treatment—the most rapid DNA expression in a high-throughput setting (up to millions of cells per minute per device). Passing cells at high speed through microfluidic constrictions smaller than the cell diameter mechanically disrupts the cell membrane, allowing a subsequent electric field to further disrupt the nuclear envelope and drive DNA molecules into the cytoplasm and nucleus. By tracking the localization of the endosomal sorting complex required for transport III protein CHMP4B (charged multivesicular body protein 4B), we show that the integrity of the nuclear envelope is recovered within 15 minutes of treatment. We also provide insight into subcellular delivery by comparing the performance of the disruption-and-field-enhanced method with those of conventional chemical, electroporation and manual-injection systems.

Intracellular delivery, the introduction of exogenous materials into cells, is essential to many studies in basic biology and biomedical research1–3. A multitude of techniques exists for cell transfection, including biological, chemical and physical methods4. Biological and chemical methods usually rely on carriers, such as viruses, vesicles, peptides or nanoparticles5–7. Physical methods primarily use membrane-disruption techniques, such as microinjection, electroporation, laser optoporation and particle bombardment, for gene delivery8–13. Although electroporation has been widely used for DNA transfection since the early 1980s, the underlying mechanism of delivery is not completely understood in nucleated mammalian cells14–19. In the electroporation process, DNA molecules accumulate and interact with the electroporëamblized plasma membrane during the electric pulse to form aggregates. Afterwards, those DNA aggregates are internalized into the cytoplasm and subsequently expressed20–26. It is unlikely that DNA plasmids navigate through the viscous and crowded cytoplasm to reach the nucleus simply by diffusion27,28. Microtubule and actin networks have been proposed to play an important role in DNA transportation within the cytoplasm, and the timescale of such processes can be hours long depending on the cell type22. The lack of detailed mechanistic understanding and the complex nature of DNA transfer between the plasma membrane and nucleus limit our ability to enhance electroporation performance. Moreover, the strong fields used in current electroporation techniques can lead to significant damage or death to cells22,26. Nanostructure-based methods have demonstrated potential for effective gene transfection by penetrating DNA-loaded nanoneedles into the cell, or by diffusion or electrophoresis through a nanostraw29–31. However, such methods typically have relatively low throughput. Moreover, the nuclear envelope rupture is not well investigated. Hence, substantial interest remains in creating techniques that can quickly and directly deliver DNA to the nucleus in a large number of cells with controllable nuclear envelope damage.

One example of a feasible strategy would involve the transient disruption of the cell plasma membrane and nuclear envelope followed by entry of the target material before resealing. Cell squeezing is a representative technique that enables delivery of a diversity of materials to numerous cell types by mechanically disrupting the plasma membrane and allowing diffusion to transport materials of interest into the cell cytosol32–35. DNA delivery is, however, more complicated because DNA must enter the nucleus to perform its function and the cytosolic delivery results in the degradation of DNA before it can reach the nucleus, as reported for microinjection31. Thus, passive diffusion of DNA is probably insufficient and active transport of the DNA to the nucleus is necessary to initiate gene expression. To address this challenge, we combine disruption of both the plasma membrane and nuclear envelope with electric fields to enhance delivery—disruption-and-field-enhanced (DFE) delivery. Recent studies have shown that moderate nuclear envelope rupture can be rapidly repaired in an endosomal sorting complex required for transport (ESCRT)-dependent manner, indicating the potential for reversible nuclear envelope rupture36–39. Here, we use a microfluidic device to create rapid mechanical deformation by cell squeezing to disrupt the plasma membrane, followed by exposing the cell to an electric field that generates reversible nuclear envelope rupture and drives the negatively charged DNA into the nucleus and cytoplasm. With this device, we show a significant increase in efficiency and speed of DNA expression, as well as rapid nuclear localization akin to microinjection. Moreover, DNA plasmids are successfully delivered to both the nucleus and cytoplasm at throughputs

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up to millions of cells per minute per device in continuous flow. We further investigate the disruption and repair of both the plasma membrane and nuclear envelope, and their relation to intracellular and nuclear delivery. The DFE system also proves useful in co-delivery of DNA, RNA and proteins.

DFE design and characterization

To explore whether addition of an electric field would be able to promote delivery of DNA after squeezing, we constructed a microfluidic device with an electric pulse zone downstream of the constriction zone (Fig. 1). Each device consisted of a set of parallel, identical constriction channels and a set of electrodes. Consistent with the existing squeeze design\(^{3,33}\), 75 parallel channels with constriction zones were etched into a silicon wafer using deep reactive ion etching. Subsequently, electrodes were incorporated into the DFE device section by anodic bonding of Pyrex patterned with electrodes (see Supplementary Fig. 1 for more details of fabrication). Based on our previous observation of the best efficiency in intracellular delivery\(^{2,33}\) with cell squeezing, the width and length of the constriction were in the ranges 4–10 μm and 10–30 μm, respectively. The length, width and gap space between each electrode were 8 mm, 60 μm and 40 μm, respectively, allowing the generation of a sufficiently high electric field (≈2 kV cm\(^{-1}\)) with low applied voltage. The duration and duty cycle of the electric pulse applied to the device ranged from 50 to 500 s and from 1 to 10%, respectively, commensurate with values commonly used for electroporation\(^{32}\).

We typically operated the DFE device at a throughput of 100,000–500,000 cells s\(^{-1}\) per chip per run (each run took 5–20 s). Each data point was the mean value of three collections. Each device could be reused 5–10 times before cell debris clogged the channels. At that point, devices were cleaned for reuse by flushing the device with 10% bleach buffer. A 50–100 μl mixture of cells and materials to be delivered was placed into the inlet reservoir and driven through the device with nitrogen pressure controlled by a regulator\(^{32}\). The exposure time of the cells in the electric field typically ranged from 10 to 100 ms, depending on the flow rate.

Many parameters governed the performance of mechanical disruption and electric delivery, including cell speed, constriction dimension, electric pulse profile, and strength and number of pulses. We first performed a number of experiments to characterize DNA transfection with the DFE technique by treating a mixture of HeLa cells and green fluorescent protein (GFP) plasmid DNA at different pulse amplitudes. After treatment, cells were incubated at 37 °C for 24 h. GFP fluorescence measurements by flow cytometry characterized DNA expression. These experiments used a device with a constriction length of 10 μm and a constriction width of 6 μm, denoted as a 10–6 DFE device. Our experimental results show that cell transfection reached above 60% and 90% when the applied amplitude increased to 8 V and 10 V, respectively (Fig. 2a, red). As a control group, we treated cells using a device with the same electric field, and cell speed but no constriction structure (squeezing), corresponding to electroporation in microfluidic devices (flow EP). With cells experiencing only an electric field, the DNA transfection efficacy reached 60% after the applied amplitude increased to 14 V (Fig. 2a, green). Both cases had similar cell viability (Fig. 2b), suggesting that mechanical disruption dramatically enhanced the DNA delivery at lower field intensities while causing negligible additional damage to the cells.

We also investigated the influence of cell speed on the transfection. Under an applied pulse of 10 V, the DNA expression decreased with increasing cell speed due to the reduced number of pulses the cell received as it travelled through the electric field (Supplementary Fig. 2). At cell speeds of ~300 mm s\(^{-1}\), cell viability and DNA expression seemed to be consistent. However, severe DNA damage at low speed and mechanical damage at high speed. The delivery efficiency of membrane-impermeable Cascade Blue-labelled 3 kDa dextran molecules

Figure 1 | Device structure and working mechanism. a, Schematic illustrating the working principle: (i) mechanical disruption of the cell membrane as the cell passes through the constriction, and (ii) the subsequent electric pulses driving DNA into the cytoplasm and nucleus through the disrupted membrane. Purple dashed lines indicate the electric field. b, Magnification of a set of identical parallel microfluidic constrictions etched into a silicon wafer (left) and a set of electrodes deposited on a Pyrex wafer (right). c, Optical image of a finished device realized by bonding silicon and Pyrex wafers. Scale bar, 1 mm. Details of the device fabrication can be found in Supplementary Fig. 1.

DFE versus microinjection, flow EP, EP and cell squeezing

To gain further insight into the mechanism of DFE delivery, we carried out a comparative study with four widely used DNA transfection techniques: microinjection (using an Eppendorf microinjector 5242), lipofection (using lipofectamine 2000 from ThermoFisher), flow EP (microfluidics-based flow electroporation) and conventional EP (using the Neon electroporation system from ThermoFisher, a common commercial electroporation tool). DNA expression was analysed using flow cytometry after treatment with each technique. EP and flow EP showed similar expression kinetics, as GFP was gradually expressed throughout 24 h after treatment (Fig. 2c,d). Seventy percent of GFP-expressing cells (cells that express GFP fluorescence after 48 h) expressed GFP between 4 and 48 h. In contrast, with microinjection and DFE, more than 80% of GFP-expressing cells had measurable expression within the first hour post-treatment, indicating that DNA transcription and translation occurred soon after treatment. The remaining 20% of ultimately GFP-expressing cells had detectable expression 1 to 4 h post treatment. Microinjection is broadly accepted as a means of facilitating direct injection of materials into the nucleus. The similar DNA expression kinetics for microinjection and DFE suggest that DNA delivered by DFE becomes accessible for transcription in the nucleus.

For the lipofection case, we found minimal GFP fluorescence in the first 4 h post treatment, and more than 95% of transfected cells expressed GFP between 4 and 48 h after treatment. Fluorescence images of GFP-expressed cells in DFE, EP and lipofection are shown in Supplementary Fig. 3. We further compared the fluorescence to live HeLa cells first dropped and then grew with increasing cell speed, indicating the dominant mechanism of delivery for this molecule switches from electric field at low speed to mechanical disruption at high speed. The difference in delivery behaviour between the 3 kDa dextran and DNA molecules further highlight the significance of the electric field effect for DNA transfection.
DNA transfection performance and expression dynamics depend on the applied electric field and methods. a, b, DNA transfection efficiency (a) and cell viability (b) at 24 h post-treatment as a function of applied electric amplitude (voltage). The introduction of mechanical disruption before electrical delivery significantly enhances the DNA transfection, while causing negligible damage to cell viability. GFP plasmid DNA transfection efficiency and cell viability were measured by flow cytometry 24 h after delivery treatment using propidium iodide staining. c, GFP expression efficiency as a function of time post-treatment. Efficiency is defined as the GFP-expressing cells over total live cells after treatment. A 10–7 chip was used for mechanical disruption (S, cell squeeze) and DFE. A pulse of 0.1 ms per 10 V at a frequency of 200 Hz was used for flow EP (microfluidic-based flow electroporation) and a single pulse of 15 ms per 15,000 V was used in EP. d, The dynamics of DNA expression was analysed by measuring differential GFP expressing at different time points after treatment. More than 80% of transfected cells expressed GFP within 1 h after treatment in microinjection and DFE. In contrast, most of the transfected cells in flow EP (60%), EP (70%) and lipofection (95%) expressed GFP 4–48 h after treatment. The number of HeLa cells in every treatment for each method is shown as well, indicating the throughput of each technique. Each data point is the mean value of triplicate experiments and error bars represent ± s.d.

To further explore the working mechanism of DFE, we directly visualized the distribution of DNA at the single-cell level using Cy3-labelled plasmid DNA (Fig. 3). Cells were first incubated with 4,6-diamidino-2-phenylindole (DAPI; ThermoFisher) and CellMask green plasma membrane stain (ThermoFisher) for nuclear and membrane staining, and then mixed with labelled DNA right before treatment with DFE, EP and squeezing. After treatment, cells were incubated in culture medium for 3–5 min and then fixed. Optical measurements were carried out using a Nikon A1R confocal microscope. When an electric pulse of 15 ms per 1,200 V, known to permeabilize cells, was applied in Neon electroporation system, sharp Cy3 fluorescence spots appeared at the plasma membrane level, indicating the absorption and accumulation of DNA on the membrane (Fig. 3b; see Supplementary Fig. 5 for additional results). This result is consistent with previous studies that demonstrate embedding of DNA into the plasma membrane. In squeezing, no fluorescence of labelled DNA was detected in the cytoplasm with the confocal microscope. In DFE, we found labelled DNA fluorescence distributed in the cytoplasm, nucleus and plasma membrane (Fig. 3c; see Supplementary Fig. 6 for additional results). The bright spots on the plasma membrane are DNA complexes formed as in conventional electroporation. Importantly, the direct visualization of DNA in the cytoplasm and nucleus further supports the hypothesis that DFE is capable of more effective delivery of DNA and indicates that the mechanism of action of the DFE system is probably distinct from electroporation or squeezing alone. These observations are further characterized by the relative fluorescence intensity profiles (Fig. 3d), which show the DNA distribution along the dashed line across the single cells in Fig. 3a–c treated by squeezing, EP and DFE, respectively. The higher fluorescence intensity in the nucleus than in cytoplasm after DFE could be attributed to: (1) degradation of DNA in the cytoplasm by the surrounding DNase and subsequent outward diffusion of Cy3 dye detached...
Figure 3 | Visualization of the delivery of fluorescence-labelled plasmid DNA to HeLa cells. After nucleus and plasma membrane staining, cells were mixed with Cy3-labelled plasmid DNA before transfection. After treatment, cells were washed with Opti-MEM and fixed using a cell fixation kit, ready for confocal imaging. a, Using only cell squeezing (S), no fluorescence-labelled plasmid DNA signal was detected in the cell. A 10–7 chip was used at a cell speed of 500 mm s\(^{-1}\). b, In EP, an electric pulse of 15 ms per 1,200 V was applied using a Neon electroporation system. DNA accumulation was found on the plasma membrane. c, In DFE, a significant Cy3 fluorescence was observed, filling the cytoplasm and nucleus. A 10–7 DFE chip was used at a cell speed of 500 mm s\(^{-1}\) with an applied electric pulse of 0.1 ms (200 Hz) at 10 V. Labelled DNA was detected in the plasma membrane, cytoplasm and nucleus. Original confocal fluorescent images are shown. d, Relative fluorescence intensity profile over the dashed line across single cells in a–c shows the labelled DNA molecule distribution after treatments of S, EP and DFE. Scale bar, 10 μm.

from the degraded DNA; or (2) more DNA trapping in the dense nucleus than surrounding cytosol as DNA transits through the cell in the electric field. Combined with our observations of rapid DNA expression, either case demonstrates that DFE delivery facilitates efficient delivery of DNA directly to the nucleus.

The ESCRT-III complex is involved in the repair of both the plasma membrane and nuclear envelope rupture\(^{36–39}\). We used HeLa cells expressing GFP-tagged charged multivesicular body protein 4B (CHMP4B), an important ESCRT-III complex subunit, to study the nuclear envelope rupture and repair in DFE. We can induce recruitment of CHMP4B–GFP at the wounding site near the nuclear envelope using microinjection (Supplementary Fig. 7). In DFE, CHMP4B–GFP formed transient foci at the site of both the nuclear envelope and plasma membrane (Fig. 4a). In EP and squeezing, CHMP4B–GFP was only recruited to the plasma membrane. CHMP4B–GFP localized to the site of the both plasma and nuclear membrane at 1–2 min right after membrane disruption to repair damage, and decreased after rescaling (Fig. 4b,c and Supplementary Fig. 8). This suggests that DFE could create reversible disruption on both the plasma plasma and nuclear envelope, a critical step for nuclear delivery.

Discussion
Intracellular delivery of nucleic acids is a challenging first step for an abundance of biological studies and applications. However, the current leading methods for DNA transfection, such as electroporation and lipofection, rely on a delayed trafficking of DNA to the nucleus. In contrast, mechanical and other permeabilization techniques that deliver DNA directly to the cytoplasm often fail to achieve nuclear penetration and subsequent expression. Here, the proposed DFE concept combines the efficacy of mechanical membrane disruption with the driving force of a field—thus potentially maintaining the robust and rapid delivery capabilities of mechanical disruption while enhancing nuclear delivery of plasmids. DFE performance is probably a nonlinear combination of mechanical disruption and electric delivery, each of which are influenced by complex sets of parameters. The choice of the buffer solution medium is a challenge. Commercially available buffers (including electroporation buffer from Neon and Eppendorf) work well for electric delivery, but not for cell-squeezing-based membrane disruption. Phosphate-buffered saline (PBS) works well for cell squeezing, but not for electric delivery owing to its high conductivity, which produces electrolytic effects including changes in temperature, pH and the chemical composition of the solution in proportion to the applied voltage. The hyposmolar buffer we used here is compatible with both the mechanical disruption and electric delivery processes. As expected, we found that hypo-osmolar buffer enlarged cell size, made the plasma membrane more susceptible to disruption\(^{40}\) and subsequently lowered the cell velocity required for mechanical disruption. The use of hyposmolar buffer also facilitated effective electric delivery\(^{41}\). Lower electric field strength for electric delivery could be used at low cell speeds to protect electrodes and avoid electrolysis.

DNA transport from the plasma membrane to the nucleus and subsequent transcription is a complex, most likely active, process that can take hours and may vary dramatically among different cell types. This process is essential in electroporation and carrier-based methods such as lipofection. We have demonstrated that DFE is able to deliver DNA directly into the nucleus by providing a driving force to move DNA across a mechanically disrupted plasma membrane. The mechanical disruption decreases the plasma membrane barrier function to achieve an enhanced electric delivery. The post-squeezing electric field might also alter the distribution of openings in the membrane\(^{42}\), to facilitate intracellular delivery. To our knowledge, these DFE experiments represent the most rapid expression of
plasmid DNA in a high-throughput setting (up to a few million cells per device per minute). The throughput could be further improved by adding more parallel microchannels on the chip or operating multiple devices in parallel. As shown in Supplementary Fig. 2, the increase of diffusion-based delivery coincided with decreasing DNA transfection when increasing cell speed; this is due to the inverse dependence between cell squeezing speed and electric field exposure. High cell speed enhances strong mechanical disruption and thus more diffusive delivery; it also leads to a shorter exposure time in the electric field, causing lower electric-field-driven delivery. Such interdependence is a limitation of the current version of the device.

The DNA expression dynamics of lipofection in Fig. 2c,d reveal that DNA transfer to the nucleus and subsequent transcription can require over 4 h in HeLa cells. DNA expression with conventional electroporation was slightly faster. There is an ongoing debate regarding how DNA migrates into the nucleus during the electroporation process. Some hypothesize that the electric pulse permeabilizes the cell membrane and electrophoresis drives DNA directly into the nucleus, while others observe that DNA first form aggregates at electropermeabilized areas of the plasma membrane during the electric pulse and then migrates towards the nucleus through a biologically active process. In our EP results, 20% of transfected cells expressed GFP within the first hour and 80% expressed throughout the next 20 h. This could be an indication that both of the aforementioned mechanisms occurred in EP. The small portion of cells that express GFP immediately after treatment may involve direct electrophoresis of DNA into the nucleus while the majority of cells that expressed GFP after 4 h must first transport the DNA to the nucleus for expression, as in the case of lipofection. Future studies could integrate a cell-trap structure in the electric field downstream of the mechanical disruption to visualize DNA migration through the mechanically induced disruption in the plasma membrane.

Recently, ESCRT-III proteins have been shown to be involved in both plasma and nuclear membrane repair. We used HeLa cells expressing GFP-tagged CHMP4B, an important subunit of the ESCRT-III complex, to monitor and measure the membrane dynamics in DFE. The recruitment of CHMP4B–GFP foci at both plasma and nuclear membrane after DFE treatment reveals that both membrane systems were disrupted and resealed, which is a critical step for nuclear delivery. In squeezing and EP, however, CHMP4B–GFP foci were observed after squeezing compared with EP and DFE. It is known that electric fields usually generate pores in the range of a few nanometres or tens of nanometres, while mechanical disruption in squeezing at high flow rates could generate larger wounds or prompt membrane plasma repair via pathways other than ESCRT-mediated processes. The recruitment of CHMP4B–GFP to both plasma and nuclear membrane wounds occurred from 1–2 min after the disruption, and nuclear membrane repair was completed by about 10–15 min post-disruption, about 5 min slower than plasma membrane resealing. Such repair dynamics are consistent with recently published reports.

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**Figure 4** ESCRT-III recruitment for plasma membrane and nuclear envelope repair. CHMP4B–GFP-expressing HeLa cells were stained with DAPI to visualize the nucleus before treatment. After treatments of S, EP or DFE, cells were incubated in culture media, and fixed with a cell fixation kit at 0.5, 1.5, 2.5, 3.5, 5.5, 10 and 15 min after treatment, and processed for both fluorescence and DIC (differential interference contrast) confocal imaging. a. Confocal images of representative cells at 1.5 min after no treatment (NC, negative control), S, EP and DFE. CHMP4B–GFP foci are visible at both the plasma membrane and nuclear envelope in DFE, but only at the plasma membrane in S and EP. Original confocal fluorescent images are shown. b,c. Number of CHMP4B–GFP foci at the plasma membrane (b) and nuclear envelope (c) after treatment of S, EP and DFE (ten cells at each data point). Each data point represents the mean value of 10 cells and error bars represent ± s.d.
DFE delivery combines microfluidic-based membrane disruption and field effects to achieve potentially greater efficacy than either individual approach. The mechanical disruption techniques, such as squeezing, have shown significant success in delivery of a variety of materials, including proteins and nanomaterials, to a diversity of cell types with minimal toxicity, but have had limited success with DNA, presumably due to ineffective nuclear delivery. In DFE, we have demonstrated a successful co-delivery of DNA plasmid, messenger RNA and protein (APC mouse IgG1, κ isotype control antibody; Supplementary Fig. 9). The results show that DNA and messenger RNA are significantly dependent on the electric field, while the protein delivery is more dependent on mechanical disruption. In mouse embryonic stem cells (mESC), we achieved DNA transfection of 19% and 36% at 10 V and 14 V, respectively, in DFE (Supplementary Fig. 10a–c). By combining mechanical disruption and electric field effects, our DFE concept has demonstrated reversible nuclear and plasma membrane disruption for both nuclear and cytosolic delivery, and is capable of co-delivering proteins and nucleic acids—characteristics that are difficult to accomplish with any of the aforementioned methods individually.

**Outlook**

Our DFE system is able to deliver DNA molecules into the nucleus and cytoplasm at high throughput with minimal cell damage. We anticipate that such direct and rapid nuclear delivery will find utility in studies of fundamental biology and biomedical applications, such as the implementation of more robust DNA transfection for cell-based therapies. Future work will focus on developing a deeper understanding of the interplay between the membrane disruptions, electric-field-enhanced transportation, and the cell response, thus facilitating effective implementation in more challenging cell types and applications. One would expect the parameter space of DFE systems to be more complex than squeezing or electroporation alone, as its efficacy relies on the synergetic interplay of relevant parameters from both techniques. Nuclear envelope disruptions in DFE can be explored further. Nevertheless, the DFE concept shows potential to enable more rapid delivery and expression compared with existing transfection technologies, and paves the way towards more targeted delivery strategies at the subcellular level.

**Methods**

**Device fabrication and experimental setup.** A silicon wafer was bonded to a Pyrex wafer to form the DFE microfluidic device. Two major steps were involved in the fabrication: (1) the fabrication of microfluidic channels on a silicon wafer; and (2) the fabrication of microfluidic electrodes on a Pyrex wafer (see Supplementary Information for more details). The device was mounted onto a holder with inlet and outlet reservoirs (more details in ref. 17). Electric pulses were generated from a function generator (Agilent 33220A) and gained through an amplifier to drive the device through the wire bonded to the electrode pads using conductive epoxy. Cells were suspended to a density of 1 × 10^6 cells ml^{-1} in a modified buffer (25 mM KC1, 0.85 mM K,H,P04, 0.3 mM KH,PO4, 36 mM myo-inositol, pH 7.2, conductivity 3.5 mS cm^{-1} at 25 °C; ref. 18) for experiments. Solutions of cells, mixed with the desired delivery material, were placed in the inlet reservoir. This reservoir was then connected to a compressed air line controlled by a pH 7.2, conductivity 3.5 mS cm^{-1} buffer. They were maintained in geneticin-containing media and FACS sorted before experiments.

**Delivery materials.** Fluorescently labelled molecules, including dextran and plasmid DNA, were mixed with cell solution at a concentration of 0.1 mg ml^{-1}. GFP plasmid DNA was used to measure the DNA transfection.

**Flow cytometry.** For quantitative analysis of cells after DNA delivery, cells were treated with trypsin 24 h after the delivery experiment and washed twice with PBS (200 μl per well in a V-bottom 96-well plate). They were then resuspended in PBS solution supplemented with 3% FBS, 1% Pluronic F-68 (Sigma) and 1 μg ml^{-1} propidium iodide (Sigma) for analysis using a LSRFortessa cell analyser (BD Biosciences).

**Lipofection.** A Lipofectamine 2000 DNA transfection kit was used to represent the lipofection technique. The DNA–lipid complex was prepared by combining 2 μl of Lipofection 2000 reagent in 100 μl of Opti-MEM medium with 2 μg of DNA plasmid in 100 μl of Opti-MEM medium, followed by 5 min of incubation at room temperature. The DNA-lipid complex solution was added to the cell sample at a ratio of 1:30. More details can be found in the product protocol (Lipofection 2000, Life Technologies).

**Microinjection.** The microinjection of DNA plasmid into HeLa cells was operated by experienced staff. Thirty cells were injected for each condition. The DNA concentration in the buffer for injection was 0.1 μg ml^{-1}. A pressure of 60 hPa and a duration of 0.2 s were used for each injection.

**Data availability.** The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary Information.

**Received 10 May 2016; accepted 27 January 2017; published 9 March 2017.**

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Acknowledgements
We thank P. Qi from the Division of Comparative Medicine at Massachusetts Institute of Technology (MIT) for performing the microinjection, Q. Chen from Richard Sherwood Lab at Brigham and Women’s Hospital for providing mouse embryonic stem cells, and X. Yin from the Jeff Karp Lab at Brigham and Women’s Hospital, Harvard Medical School for providing human embryonic stem cells. We thank I. Poser from Tony Hyman Lab at Max Planck Institute of Molecular Cell Biology and Genetics for supplying the CHMP4B-GFP HeLa cells. The assistance and expertise of G. Paradis and personnel in the flow cytometry core at the Koch Institute and the Microsystem Technology Laboratory at MIT are highly acknowledged. This research was supported by National Institutes of Health (R01GM101420-01A1), and device fabrication was performed at the Microsystem Technology Laboratory at MIT. M.P.S. was supported by the Swiss National Science Foundation through the advanced postdoc mobility fellowship P300P3_151179. M.P.S. acknowledges support from a Keith Murdoch Fellowship via the Australian Association, a Life Sciences Research Foundation Fellowship sponsored by Good Ventures, and a Broadnext10 Catalytic Steps funding gift from the Broad Institute.

Author contributions
X.D., M.P.S., A.S., R.S.L. and K.F.J. designed the research, X.D. and M.P.S. performed the experiments and X.D. fabricated the devices. X.D., M.P.S., A.S., J.C.W., R.S.L. and K.F.J. wrote the data. X.D., M.P.S., A.S., J.C.W., R.S.L. and K.F.J. wrote the article.

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How to cite this article: Ding, X. et al. High-throughput nuclear delivery and rapid expression of DNA via mechanical and electrical cell-membrane disruption. Nat. Biomed. Eng. 1, 0039 (2017).

Competing interests
A.S., R.S.L. and K.F.J. have a financial interest in SQZ Biotechnologies.