Localized electroporation with track-etched membranes

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In PNAS, Cao et al. (1) employ track-etched membranes for the intracellular delivery of various molecular cargoes via electroporation in both adherent and suspended cells. While the concept of utilizing such membranes for electroporation is not novel (2, 3), its extension to suspended cells and the wide parametric space covered in the article are noteworthy. The objectives of this letter are to correct a minor lapse in the article's introduction, as well as to put the results in a broader context. In ref. 1, the authors cite that the delivery efficiency of a plasmid, using track-etched membranes and localized electroporation (2), was less than 50% (reference 16 in ref. 1). However, an efficiency >70% was reported in ref. 3. The enhancement was made possible through insights drawn from multiphysics modeling of the device architecture and pore evolution as a function of electroporation parameters (3, 4). We contend that the model predictions are equally applicable to the study by Cao et al. (1). Specifically, the simulations reported in ref. 3 predict the existence of an intermediate voltage where the transport of macromolecules is optimized. An insufficient number of large pores is created at low voltage amplitudes, whereas at very high values many small pores are created that do not expand to larger radii. As both extremes are unfavorable to macromolecular delivery, there is an optimal voltage in between. We had validated this prediction by delivering an mCherry encoding plasmid in MDA-MB 231 and HT 1080 cells at various voltages between 0 and 40 V (see figures 3 and 4 in ref. 3), with 30 V being the optimal case. It is interesting to note that a similar conclusion is borne out from the data in the study by Cao et al. (figures 2 A and C and 4 A and C in ref. 1), in which the transfection efficiency of mCherry messenger RNA (mRNA) and green fluorescent protein-encoding plasmid is optimal for an intermediate voltage of 20 V in HeLa and 30 V in Jurkat cells. Further, the amount of mRNA or plasmid delivered, as indicated by fluorescence intensity in figure 2 B and D in ref. 1, appears substantially nonuniform: Some cells are much brighter than the others. In this context, the simulations also predict that the variation in the delivered amount can be reduced by increasing the membrane tension of the cells. In experiments, this can be readily achieved by increasing the osmolarity of the electroporation buffer (5), as we had shown in the delivery of a large fluorescent protein (figure 6 in ref. 3).

The wide variety of macromolecules delivered using track-etched membranes and localized electroporation (1–3) combined with fabrication simplicity should allow for an easy adoption of the technology by the biological research community. The study by Cao et al. (1) also shows that more complex delivery formats, such as nanostraws (6), needles (7), or single nanochannels (8), may be sufficient but not necessary tools for intracellular delivery. Moreover, it shows that a comparative study of transfection efficiency as a function of cell type and macromolecule is lacking and needs further exploration. Likewise, in addition to basic viability protocols, more sophisticated studies investigating the medium- and long-term transcriptomic, genomic, and epigenomic effects of different electroporation technologies should be pursued.

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