Mechanistic analyses of polymer/lipid-based gene transfection processes through membrane integrity assay using proton sensing transistor

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Introduction

The endosomal membrane is a major barrier for efficient transfection and endosome escape has become known as a crucial step in the delivery of nucleic acids. Previous research revealed distinct reagent-mediated membrane disruption mechanisms (Figure 1): the formation of small pores allowing protons to pass biological membranes and the permeabilization of large molecules such as LDH through amphiphilic translocation [1]. Here, we investigate the endosome escape mechanism of commercial transfection reagents by assessing the membrane permeability of different molecules. The endosome escape profile is also assessed using a CLSM-based biological assay. Understanding these mechanisms is expected to contribute to better design of nucleic acid carriers.

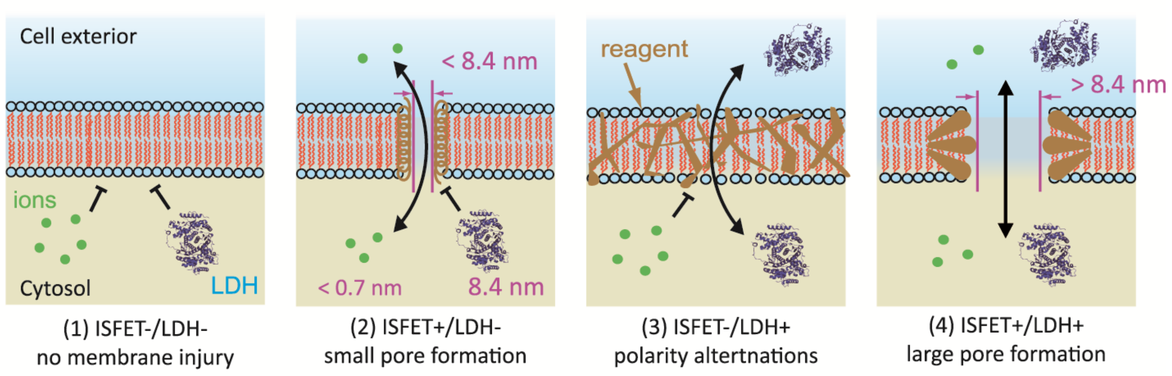


Figure 1. Proposed mechanisms for membrane disruption by transfection reagents. Mechanisms can be distinguished by assessing the membrane permeability of different molecules. Adapted from Goda *et al.*[1]

Materials and Methods

HepG2 cells were seeded on an ion-sensitive field-effect transistor (ISFET) chip. Lipofectin or *in vivo*-jetPEI were diluted in appropriate buffer at different ratios. The cells were superfused with alternating cycles of buffer solution while measuring the gate potential at the sensing area of the ISFET. LDH leakage was assessed with a commercial LDH kit according to the manufacturer's instructions. HepG2 cells were transfected with Cy5-labeled DNA using Lipofectin. Early and late endosomes were stained with CellLight GFP. Fluorescence microscopy images were taken with CLSM and endosome escape was evaluated at different time points.

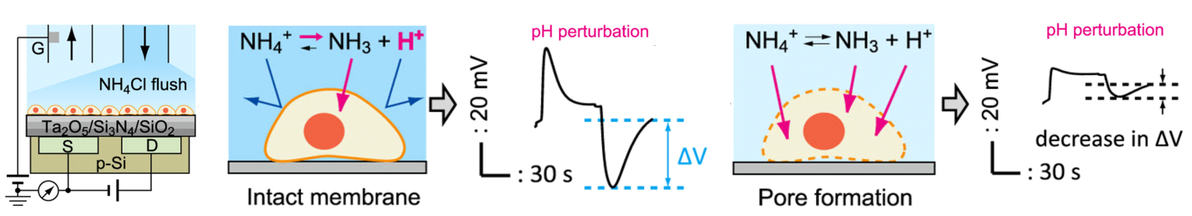


Figure 2. By measuring transient changes in pH (ΔV) in the local microenvironment using an ion-sensitive field-effect transistor (ISFET), the permeability of the membrane for protons can be assessed. The formation of membrane pores causes a decrease in ΔV. Adapted from Goda *et al.*[1]

Results

ISFET assay: Both reagents cause proton leakage at pH 5.5

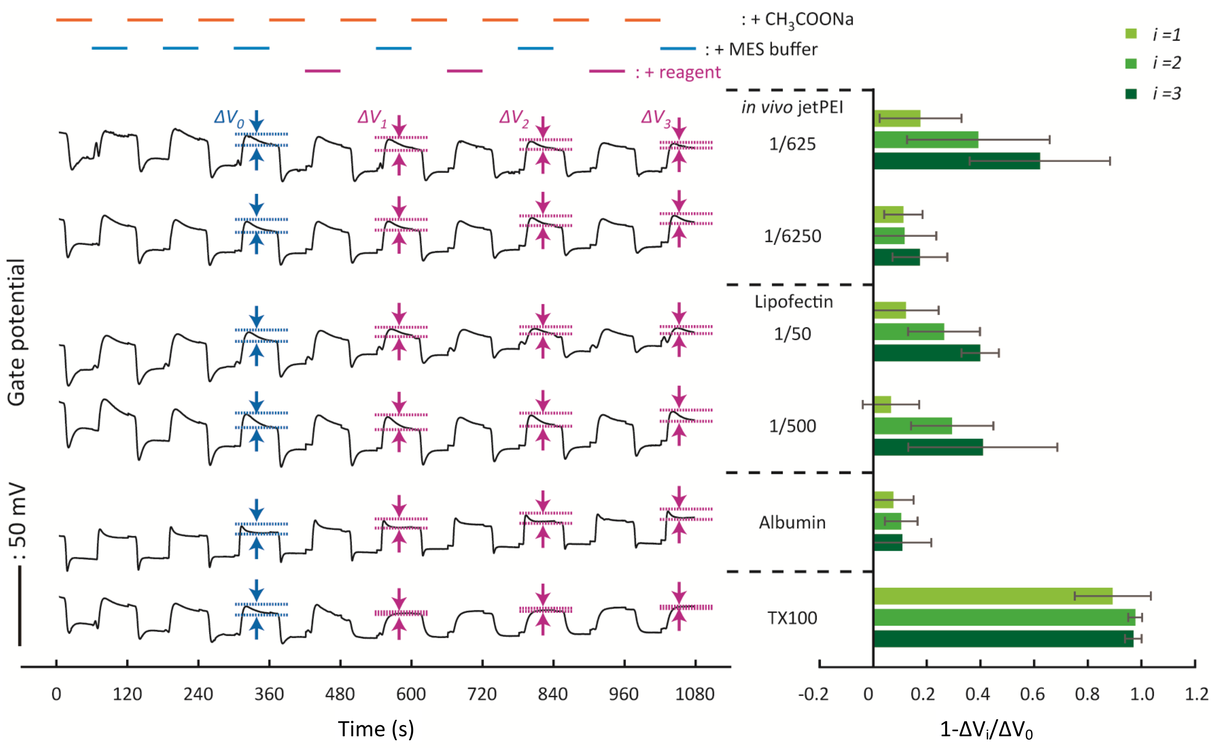


Figure 4. ISFET measurements of cells superfused in 60-second cycles with MES buffer with and without sodium acetate, alternated with superfusions of transfection reagent dilutions in MES buffer at pH 5.5. The data show that at this pH, both polymer-based transfection reagents (In vivo Jet-PEI) and lipid-based transfection reagents (Lipofectin) induce proton leakage at high concentrations (10x working concentration). At normal working concentrations, only Lipofectin shows clear indications of proton leakage. Data is presented as average relative difference between the baseline peak (ΔV_0) and subsequent peaks (ΔV_i) after superfusion of reagent (n=3). Error bars represent S.D.

ISFET assay: No proton permeability at pH 7.4

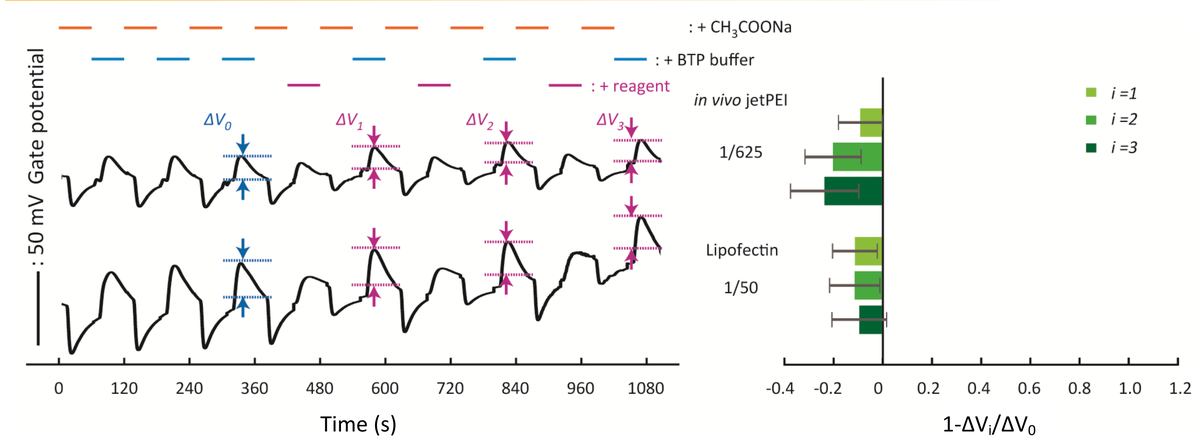


Figure 3. ISFET measurements of cells superfused in 60-second cycles with BTP buffer with and without sodium acetate, alternated with superfusions of transfection reagent dilutions. Data is presented as average relative difference between the baseline peak (ΔV_0) and subsequent peaks (ΔV_i) after superfusion of reagent (n=3). Error bars represent S.D.

Only polymer-based reagents at pH 5.5 cause LDH leakage

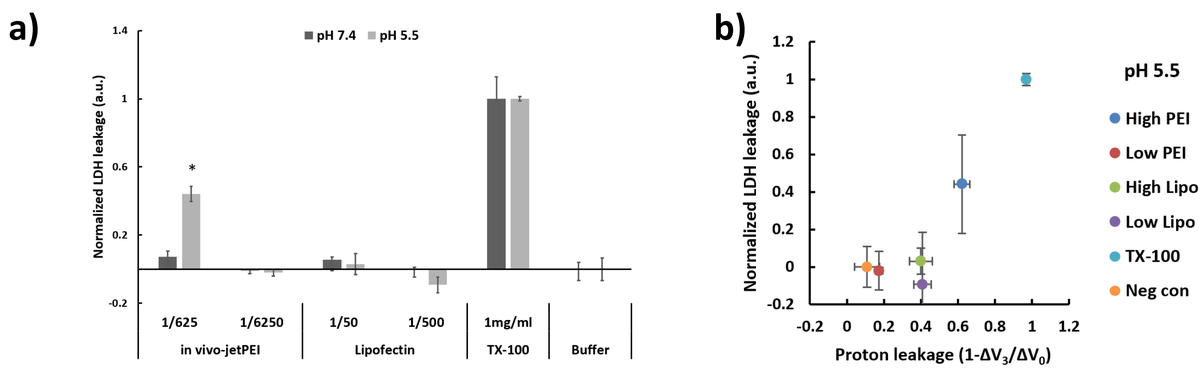


Figure 5. (a) LDH leakage after incubation with polymer and lipid-based transfection reagents. Data is presented as average relative LDH leakage normalized to positive and negative control (n=3). Error bars represent S.D. (b) Scatter plot of the data from both assays at pH 5.5. Error bars represent S.D.

Lipofectin shows gradual endosome escape

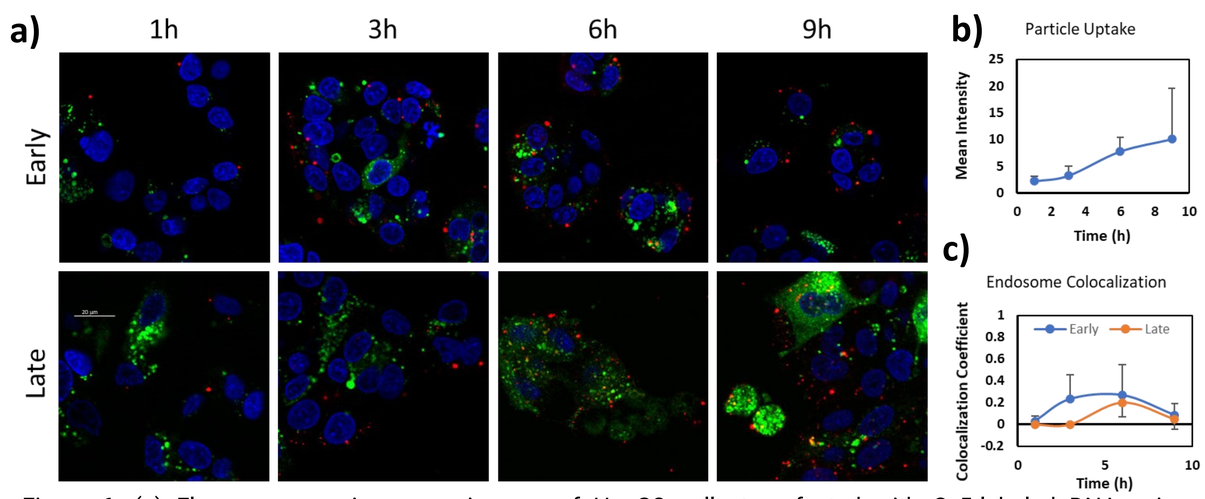
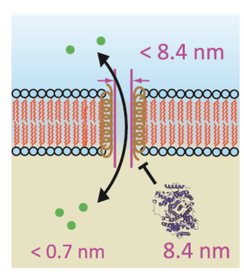


Figure 6. (a) Fluorescence microscopy images of HepG2 cells transfected with Cy5-labeled DNA using Lipofectin. Early and late endosomes are stained using CellLight GFP. (b) Cellular uptake of labeled DNA. Error bars represent S.D. (c) Colocalization of Cy5 and GFP determined with ZEN software. Error bars represent S.D.

Discussion and Conclusion

Our data indicate that both types of transfection reagents have pore-forming activity at endosomal pH, while there is no such activity at pH 7.4. The pores formed by polymer-based reagents appear to be larger than those formed by lipid-based reagents. This is in line with findings by Rehman *et al.* who found that polymer-based carriers quickly release their payload from the endosome whereas lipid-based reagents display a more gradual transfer of the cargo [2]. CLSM data confirms that Cy5-DNA delivered by Lipofectin gradually escapes the endosome.

Lipid-based:
Lipofectin
Small pores
< 8.4 nm



Polymer-based:
In vivo Jet-PEI
Large pores
> 8.4 nm

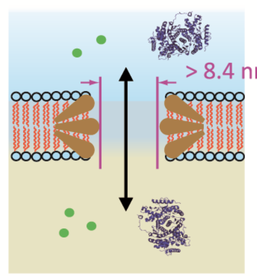


Figure 6. Proposed mechanisms for endosome escape by commercial transfection reagents. Based on the LDH and ISFET data, the pores created by polymer-based reagents appear to be larger (> 8.4 nm) than those made by lipid-based reagents (< 8.4 nm). Adapted from Goda *et al.*[1]

References

1.Tatsuro Goda, et al., *Langmuir*, **2019**, 35(24), 8167-8173.
2. Zia ur Rehman, et al., *ACS Nano*, **2013**, 7(5), 3767-3777.

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