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Efficient Electroporation of DNA and Protein into Confluent and Differentiated Epithelial Cells in Culture

Ami A. Deora[†], Fernando Diaz[†], Ryan Schreiner, and Enrique Rodriguez-Boulan^{*}

Margaret M. Dyson Vision Research Institute, Department of Ophthalmology, Weill Medical College of Cornell University, New York, NY 10021, USA

Abstract

Electroporation-mediated delivery of molecules is a procedure widely used for transfecting complementary DNA in bacteria, mammalian and plant cells. This technique has proven very efficient for the introduction of macromolecules into cells in suspension culture and even into cells in their native tissue environment, e.g. retina and embryonic tissues. However, in spite of several attempts to date, there are no well-established procedures to electroporate polarized epithelial cells adhering to a tissue culture substrate (glass, plastic or filter). We report here the development of a simple procedure that uses available commercial equipment and works efficiently and reproducibly for a variety of epithelial cell lines in culture.

Keywords

apical; basolateral; Caco-2; electroporation; MDCK; polarized epithelium; RPE

Studies with polarized epithelial cell lines in culture have provided tremendous insights into the fundamental mechanisms underlying the establishment of epithelial polarity, vectorial transport/secretion of metabolites and polarized trafficking of plasma membrane components (1). Key tools in such studies are procedures to introduce genes, proteins and small molecules to evaluate epithelial properties and functions. Cationic lipids or electroporation that creates transient holes in the plasma membrane operates efficiently in cells kept in suspension culture and in sparse cultures of adherent cells but is not efficient in fully polarized epithelial monolayers grown on tissue culture substrates (2–11). As epithelial monolayers plated on glass, plastic or filter substrates require very long times to fully polarize and differentiate, e.g. 4–5 days for the prototype kidney cell line Madin–Darby kidney cells (MDCK) (12–14), 3–4 weeks for intestinal epithelial (Caco-2) cells (15) and 6 weeks or more for the human ARPE-19 cell line or primary human retinal pigment epithelium (RPE) cultures (16,17), the expression of the transfected gene may be lost or may cause unwanted disruptions in the development of polarity. Microinjection of plasmid DNA into the nucleus is an excellent method to synchronize protein trafficking across the cell (18,19) but is very laborious and inefficient and cannot be applied to heavily pigmented cells

such as RPE. Adenoviral vectors are currently the method of choice to introduce genes into fully polarized epithelial cells (20–22), but this procedure is limited to DNA, depends on the expression of adenovirus receptors at the apical cell surface, variable in different epithelial cells (Diaz et al., manuscript in preparation), and is hindered by the time and expense required to build the viral vector. Thus, a method to efficiently transduce fully polarized epithelial monolayers in culture is highly desirable.

An electroporation procedure for polarized epithelial cells in culture should ideally fulfill the following requirements: (a) preserve the integrity, architecture or functional properties of the epithelium and the individual cells, (b) be efficient and reproducible, (c) allow efficient penetration of different types of macromolecules and (d) allow monitoring of basic epithelial properties at various times before and after electroporation. Various attempts have not been successful in developing a simple and reproducible procedure for electroporation of polarized epithelial cell (5–8,10,23). Gharthey-Tagoe et al. (5,23) utilized a special cuvette and an innovative electrode design to deliver electric pulses that was reportedly efficient in transfecting macromolecules into polarized intestinal epithelial monolayers (Caco-2 and T84). However, this electroporation technique resulted in poor recovery of functional tight junctions, as measured by transepithelial resistance (TER) and cannot be currently utilized, as commercial distribution of the special cell cuvette has been discontinued.

Here, we report the development of an optimized electro-poration protocol to deliver molecules into fully polarized epithelial monolayers grown on filters. The protocol works efficiently and reproducibly for the transfection of DNA and protein in MDCK, ARPE-19 and Caco-2 cells, preserves all crucial features of these polarized cells, can be extended to a wide range of cell types including valuable human specimens and utilizes readily available commercial equipment without any modification.

Results

Optimization of buffer and electrical parameters for electroporation of MDCK cells

We empirically tested several parameters reported as important for electroporation (5,8,23–25), using commercial www.traffic.dk electroporation equipment recommended for *in vivo* gene delivery (Nepagene). For the initial experiments, we chose MDCK cells because they polarize in just 4–5 days, rather than the several weeks required by retinal ARPE-19 and intestinal Caco-2 cells and a plasmid encoding a basolateral protein, CD147–green fluorescent protein (GFP), at a concentration of 10 µg/mL in four different solutions: serum-free DMEM (Cellgro, Herndon), PBS (Cellgro), hypoosmolar electroporation buffer (HEB) (KCl 25 mM, KH₂PO₄ 0.3 mM, K₂HPO₄ 0.85 mM, myo-inositol 90 mOsmol/kg, pH 7.4) and isoosmolar electroporation buffer (IEB) (identical to HEB except that the concentration of myo-inositol was 280 mOsmol/kg). HEB and IEB have potassium concentrations 10 and 5 times higher and ionic strengths ~10× lower than PBS and DMEM, respectively. Low ionic strength and neutral pH have been shown to be important for efficient electroporation (26). Potassium concentrations around 20 mM depolarize the plasma membrane of MDCK cells and increase its conductance (27), facilitating pore formation. Electrical parameters tried previously with different electrode designs (9,23,28) include combinations of shorter high-voltage (HV) pulses (single pulse, 250–500V, 0.15–0.3 milliseconds) with longer low-

voltage (LV) pulses (5–50 pulses, 5–50V, 5–50 milliseconds). We tested several combinations of HV and LV pulses and different number of low-voltage pulses as described in Table S1. We empirically selected a combination that provided the most efficient electroporation while preserving monolayer integrity: 1 pulse, 300V, 0.15 milliseconds followed by two rounds of 15 pulses, 25V, 25 milliseconds with 100-millisecond interval between pulses. HV pulse above 300V and for more than 150 microseconds proved to be detrimental to the integrity of the cell monolayer.

Figure 1 shows the electroporation efficiency achieved with the electrical parameters and the four buffers mentioned above. We found that IEB provided significantly higher electroporation efficiency of CD147–GFP than the other buffers. Similar results were obtained with various other plasmids used in this study (data not shown). Interestingly, addition of 20 mM KCl to DMEM did not increase electroporation efficiency over just DMEM (data not shown). This finding suggests that in addition to high potassium level, physiological osmolarity is also a critical determinant of optimal electroporation.

Optimization of plasmid concentration for electroporation efficiency and cell viability

The yield of positive cells increased with increasing concentrations of plasmid DNA (range 5–30 $\mu\text{g/mL}$) in IEB (Figure 2A): 5 $\mu\text{g/mL}$ of complementary DNA (cDNA) encoding CD147–GFP did not yield any positive cells, whereas 10–30 $\mu\text{g/mL}$ yielded a large fraction of positive cells around the area where the electrode was placed. Similar results were obtained with neural cell adhesion molecule (NCAM)–GFP, p75–GFP and glycosylphosphatidyl inositol (GPI)–yellow fluorescent protein (YFP) plasmid DNA (data not shown). We found that the expression level was sustained for up to 72 h (Figure 2B). Using this protocol with a plasmid concentration of 10–15 $\mu\text{g/mL}$ did not result in any alteration of TER 1 and 24 h after electroporation (Figure 2C). We calculated electroporation efficiency in the field under the electrode by determining the percentage of transfected cells in 8–10 fields at 63 $\times$ magnification. As shown in Table 1, 30 $\mu\text{g/mL}$ of plasmid resulted in $49.69 \pm 12.02\%$ (mean $\pm$ SD) transfection efficiency, while 10 $\mu\text{g/mL}$ of plasmid resulted in $24.51 \pm 5.11\%$ electroporation efficiency. As determined morphologically with 4',6-Diamidino-2-phenylindole (DAPI) nuclear staining and tight junction (TJ) staining with Zonula Occludens 1 (ZO-1) antibodies, the monolayers remained intact at plasmid DNA concentrations of 10 $\mu\text{g/mL}$, but some cell death was observed after 48–72 h at a plasmid DNA concentration of 30 $\mu\text{g/mL}$. Hence, as a compromise between electroporation efficiency and monolayer integrity, we used 10 $\mu\text{g/mL}$ of plasmid DNA for all subsequent experiments.

Electroporation results in much higher transfection efficiency than cationic lipids

As we completed the optimization of our electroporation protocol, we compared its transfection efficiency with that of two popular cationic lipids, Lipofectamine 2000TM and EffecteneTM, used according to an established protocol (11). Figure 3A depicts images of entire 12-mm transwell filters covered with confluent MDCK cells expressing transfected CD147–GFP (green) and their nuclei stained with DAPI (pseudocolored red). Transfection efficiency was much higher in the area close to the electroporation electrode (Figure 3A, electroporation panel, arrows) than in monolayers transfected with LipofectamineTM and

Effectene™, as shown by the magnified images in Figure 3B. The results are shown quantitatively in Figure 3C, which compares the average GFP–DAPI ratio of 10 different microscopic fields within the transfection-positive area of electroporated cells with 10 random fields from monolayers transfected with Lipofectamine™ or Effectene™ (Figure 3C). Electroporation resulted in a GFP–DAPI ratio of around 20 times higher than that of cationic lipids in this experiment.

Plasmid purity did not affect electroporation efficiency

The presence of endotoxin in the plasmid preparation is known to decrease the transfection efficiency of cationic lipids (29) as well as of the Amaxa™ nucleofection protocol (manufacturer's manual; Amaxa Nucleofector system). The effect of endotoxin might be on transfection efficiency or cell viability after transfection, or both. Interestingly, electroporation efficiency was identical with plasmids purified with either endotoxin-free kits or with regular miniprep kits (data not shown). Thus, the purity of the plasmid did not determine the outcome of the electroporation protocol.

Electroporated markers are correctly targeted in various epithelial cell lines

We next determined whether our electroporation protocol resulted in the targeting of well characterized apical and basolateral markers to their correct locale at the cell surface. To this end, we used plasmids encoding the apical markers p75–GFP (18,30) and GPI–YFP (20) and the basolateral markers CD147–GFP (4) and NCAM–GFP (19,31). Examination of the monolayers by confocal microscopy showed that overexpressed p75–GFP and GPI–YFP were targeted to the apical membrane (Figure 4 A), whereas CD147–GFP and NCAM–GFP were targeted to the basolateral membrane (Figure 4B). Electroporation of a *trans* Golgi network (TGN)-resident protein, sialyltransferase tagged with red fluorescent protein (RFP) (ST-RFP), also resulted in correct targeting (Figure 4C). Moreover, co-electroporation of CD147–GFP and p75–RFP resulted in the correct localization of both cargo proteins (Figure S1). We were also able to detect the expression of electroporated p75–GFP in 12-mm transwell filters biochemically, i.e. by Western blot (Figure 4D). This experiment showed that the expression of p75–GFP varied within a 1.85-fold range in nine independent filters. Future improvements in electrode design should result in improvements in the variability of protein expression and in efficient transfection of a larger fraction of the monolayer surface area.

Some epithelial cell lines, e.g. the human intestinal line Caco-2 and the RPE cell line ARPE-19, take 3–6 weeks to fully polarize (15,17). As a result, studies involving transient gene expression in these polarized cells are very difficult. However, electroporation of ARPE-19 monolayers grown on laminin-coated filters for 6 weeks resulted in efficient expression of CD147–GFP and its correct targeting to the apical domain (Figure 5A,B) (32). In these cells, the endogenous lactate transporter monocarboxylate transporter 1 (MCT1), which uses CD147 as a chaperone for transport to the apical surface in both MDCK and RPE cells (33), was also normally polarized to the apical surface. Finally, we were also able to successfully electroporate polarized Caco-2 cells with CD147–GFP plasmid DNA (Figure S2 and Table S2). In contrast to MDCK cells, we did not observe any plasmid-concentration-dependent increase in transfection efficiency in Caco-2 cells; moreover, the

transfection efficiency was somewhat lower (~20%) than what we observed in polarized MDCK cells. This suggests that the electroporation outcome will vary depending on the cell type under study.

Electroporation-mediated protein delivery

Last, we determined that our electroporation protocol resulted in successful delivery of Alexa-labeled antibodies (1 μ M in IEB) into MDCK cells (Figure 5C). This method should allow the study of the effect of, e.g., blocking antibodies and peptides on specific epithelial functions.

Discussion

We have developed a simple, efficient and reproducible electroporation procedure to introduce DNA or protein into polarized epithelial cells in culture. Various other groups have made attempts to electroporate adherent cells (5–8,10,23), but, to date, no simple and reproducible method is available. Gharthey-Tagoe et al. (5,24) have reported the successful electroporation of polarized Caco-2 and T84 monolayers. However, the authors report that their electroporation protocol compromises the function of tight junctions for up to 6 h; in contrast, the electroporation protocol reported here is less disruptive for the monolayer as it allows recovery of TER within 1 h. Unfortunately, we were unable to compare our electroporation protocol with the one used by Gharthey-Tagoe et al. because the electrode they utilized has been discontinued by its commercial distributor. Nevertheless, this study provided very useful clues on the optimal electrical parameters to be used in our experiments, thus providing an important contribution toward the development of an efficient and reproducible electroporation method.

The procedure we developed optimized the use of widely used commercially available electrode. It has unparalleled ease of operation with minimal experimental manipulation. Important requirements for the success of electroporation are the choice of electroporation buffer, electrical parameters and the optimal plasmid concentration. The optimal electroporation buffer was chosen after an extensive survey of the literature and systematic empirical observations. Previous studies underscored the role of high concentration of potassium in success of electroporation. Our studies suggest that in addition to high potassium concentration and low ionic strength (25,27), normal osmolality (300 mOsm/L) is also an important determinant of a successful electroporation method. By preventing excessive swelling of the electroporated cell, normal osmolality may contribute to cell survival after electroporation. After empirically testing different combinations of electrical parameters suggested by previous studies (9,23,28), we settled for a combination of HV and LV pulses that provided maximal transfection efficiency with minimal monolayer disruption. Additional experimentation led to the establishment of a plasmid concentration (10 μ g/mL) that maximized transfection efficiency and cell viability. The efficiency of our transfection protocol does not seem to require endotoxin-free plasmid preparation, which presents a distinct advantage in cost over cationic lipid-based transfection methods.

Levels of expression achieved at 1–3 days after electroporation are more than sufficient for most optical microscopy and Western blot assays. Our electroporation protocol can be potentially manipulated and adapted to different experimental needs. Utilizing different plasmid DNA concentrations and/or incubation times after electroporation can modulate changes in expression levels of the transfected gene. TER and staining of ZO-1 demonstrated preservation of the monolayer within 72 h after electroporation. This feature has never been so successfully preserved in previous studies. At later time-points, small patches of dead cells started to appear but only with the higher plasmid concentrations. Polarized sorting of apical and basolateral markers was performed efficiently by electroporated monolayers of MDCK, ARPE-19 and Caco-2 cells. The lipid-raft-associated protein GPI-Gly-YFP and the non-raft-associated transmembrane protein p75-GFP are expressed mostly at the apical surface as previously reported (18,20). The two markers CD147 and NCAM showed their previously reported basolateral localization in MDCK cells (4,19). CD147 is known to exhibit reversed apical polarity in RPE cells (32). Electroporation of CD147-GFP in ARPE-19 resulted in apical targeting of CD147. Even when two different markers were expressed by co-electroporation, each of them localized to its correct locale at the plasma membrane. Studies involving introducing blocking antibodies and peptides in polarized epithelia cells also would be possible with our electroporation protocol. Thus, we have developed a simple and powerful technique for transfecting macromolecules into confluent, fully polarized epithelial cells by means of electroporation, with minimum disruption to the epithelial monolayer. This method allows the study of the expression of genes when the epithelium has acquired its fully differentiated properties, a process that may take from 5 days to several weeks in different epithelial cell types. This method circumvents the problems presented by transfection of genes into epithelial cells in suspension or in subconfluent cultures, which may prevent the normal differentiation of the epithelial monolayer. The commercial equipment we have used requires a one-time investment and no additional expensive reagents. This technique will benefit epithelial cell biologists seeking to assess the role of any given molecule in a differentiated epithelium. We envision further applications in fields such as neurobiology, developmental biology and stem cell biology, where delivery of molecules needs to be performed with minimal or no cell loss while preserving the differentiated state of the cells.

Materials and Methods

Cell cultures

MDCK II cells were maintained in DMEM (Cellgro) supplemented with 10% FBS (ICN, Aurora, OH, USA), 1% glutamine and 1% penicillin–streptomycin at 37°C in 95% air/5% CO₂ atmosphere. MDCK cells were plated on 12-mm polycarbonate or polyester transwell filter units (0.4-μm pore size) (Costar) at a density of 250 000–300 000 cells per filter and cultured for 5 days to allow development of polarity. Medium was changed every other day. Filters exhibiting a TER around 80–100 Ωcm² were used for electroporation and immunofluorescence analysis. ARPE-19 cells (ATCC), a spontaneously arising human RPE cell line, were grown in Chee's essential medium containing 1% bovine retinal extract on laminin (BD Biosciences, CA, USA) coated transwell filters for 6 weeks to allow maximal development of polarity (4). The medium was changed twice a week. After 6 weeks, TER of

ARPE-19 cells was 50 Ωcm^2 . In our studies, we used a clone of Caco-2 (C2BBE1) obtained from ATCC. These cells were grown in DMEM supplemented with 10% FBS and 0.01 mg/mL transferrin (Sigma). C2BBE1 cells were grown on polycarbonate filters for 21 days to allow full development of polarity and differentiation (15).

TER measurements

TER was measured with an EVOM Voltohmmeter (World Precision Instruments). Resistance was assessed right before electroporation and 1 and 24 h after electroporation. Recorded values, after subtraction of values of empty filters, were multiplied by the filter area (1.13 cm^2 for the 12-mm filters) to express them as Ωcm^2 (12).

Plasmids and protein used for transfection

cDNAs encoding apical and basolateral plasma membrane proteins were used for the electroporation studies. Basolateral proteins were represented by CD147-GFP (4) and NCAM-GFP (19) and apical proteins by p75-GFP (18), p75-RFP and GPI-GFP (20). Sialyltransferase's N-terminal 37 amino acids encoding a TGN retention signal tagged to the monomeric RFP (ST-RFP) was constructed and used as a TGN marker (34). Plasmids were purified with various commercial kits, endotoxin-free maxiprep (Qiagen), Maxiprep (Marlingen Biosciences) and Miniprep plasmid purification kit (Promega), according to the manufacturers' directions. Goat anti-rat Alexa 488 (Invitrogen) was used to study protein delivery.

Electroporation apparatus and conditions

Plasmid DNA was diluted to concentrations ranging from 5 to 30 $\mu\text{g/mL}$ in the following medium and buffers: serum-free DMEM (Cellgro), PBS (Cellgro), HEB and IEB (Eppendorf Westbury, NY, USA). The components of HEB are KCl 25 mM, KH_2PO_4 0.3 mM, K_2HPO_4 0.85 mM and myo-inositol 90 mOsmol/kg, and IEB has the same components and concentration as those of HEB except that the concentration of myo-inositol was 280 mOsmol/kg.

An electrode with two plates (7 $\times$ 19 mm with a 5-mm gap) and two legs per plate (catalog number CUY512-5; Nepagene; distributed by Protech International, Inc.) was connected to an ECM 830 square-wave electroporation system with digital interface (BTX Instrument Division, Harvard Apparatus, Inc.). Media were aspirated off the apical chambers of each transwell and replaced by 300 μL of DNA-buffer mix. The electrode was brought down onto the filter until it made contact with the buffer, usually at 1–2 mm above the filter. A combination of HV and LV pulses was used. The electrode was cleaned with alcohol before changing to a different plasmid. We did not detect any cross-contamination of plasmids between the filters. The pulser settings for electroporation of plasmids and proteins were as follows:

HV: 300V, 150 microseconds, 1 pulse.

LV: Two rounds of 25V, 25 milliseconds, 15 pulses with 100-millisecond interval between pulses.

The entire procedure was carried at room temperature in a sterile tissue culture hood.

Right after pulsing the last filter, media were replaced in both chambers and the cells were incubated at 37° C.

Immunofluorescence microscopy and quantification

Cells were fixed for 20 min at room temperature in 4% freshly prepared paraformaldehyde in PBS and quenched with 50 mM NH₄Cl in PBS containing 1 mM CaCl₂ and 1 mM MgCl₂ (PBS/CM). Cells were either permeabilized with 0.075% saponin or 0.1% Triton-X-100. To determine the junctional integrity, we used rat (Chemicon) (1:400 dilution) or rabbit (Invitrogen) (1:500 dilution) antibodies against the tight junction protein ZO-1. For ARPE-19 cells, we used rabbit polyclonal antibody against MCT1 as an apical marker (33). Secondary antibodies used were tagged with Alexa Fluor 488, Alexa Fluor 568 or Alexa Fluor 633 (Invitrogen). DAPI was used to stain the nucleus. Images were obtained on a laser scanning confocal microscope (Leica TCS SP2) with 20×, 40× or 63× oil objectives. Serial *x-y* (*en face*) and *x-z* sections (top to bottom) were collected and processed by LCS software (Leica) and Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA, USA).

To compare transfection efficiencies, cells were transfected with a plasmid encoding CD147–GFP by electroporation as mentioned above or with Lipofectamine 2000™ (Invitrogen) or Effectene™ (Qiagen) as described previously (11). Entire filters were imaged using the slide scan feature of METAMORPH imaging software version 7 (Universal Imaging, Downingtown) using a Zeiss Axiovert 200 (Carl Zeiss) inverted microscope equipped with a motorized stage at 10× magnification. For quantification, 10 images of positive fields per sample were obtained using a Nikon inverted microscope (TE 300; Nikon) and a charge-coupled device camera (ORCA II ER from Hamamatsu) with a 20× objective. Acquisition and intensity measurements of the images were performed using METAMORPH. Values of empty fields (GFP or DAPI) were used for background correction. A ratio of GFP–DAPI intensity was calculated to assess the efficiency of each protocol. Arbitrary fluorescence units (GFP–DAPI) were plotted. Graphs were plotted with Graphpad prism software (Graph Pad Prism Software, Inc.).

Western blot analysis

We performed Western blot on polarized MDCK cells grown on 12-mm transwell filters, 24 h after electroporation with p75–GFP plasmid. The filters were excised and lysed in 1% Triton-X-100 lysis buffer containing protease inhibitor cocktail. Twenty micrograms of the total protein was separated by SDS-PAGE, blotted onto a nitrocellulose filter, and analyzed for p75–GFP expression by Western blot analysis with anti-GFP antibody (Abcam, Cambridge, UK). Quantification of the p75–GFP protein band was performed using SCION IMAGE software (Scion Corp., Frederick, MD, USA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Optimization of buffers for efficient electroporation

Electroporation was performed in polarized monolayer of MDCK cells with 10 $\mu\text{g/mL}$ of CD147–GFP plasmid in various buffers: DMEM, PBS, HEB and IEB. A representative image obtained with laser scanning confocal microscope with 20 $\times$ objective clearly suggests that IEB provided optimal environment for effective electroporation. Nuclei were stained with DAPI. Bar: 20 μm .

Figure 2. Determination of optimal plasmid concentration and TER

A) MDCK cells were polarized for 5 days on polyester–polycarbonate filters and then subjected to electroporation using different concentration of CD147–GFP plasmid DNA. Concentration range of 5–30 µg/mL of DNA diluted in IEB was used. 10 to 30 µg/mL of DNA was highly effective concentration in yielding more than 60% cells positive around the electrode. Electroporation with the concentration of 5 µg/mL of CD147–GFP DNA did not result in any positive transfected cells. Images were obtained by laser scanning confocal microscope (LSCM) with 20× objective. Bar: 20 µm. B) Expression of CD147–GFP protein was robust after 24 h and was sustained till 72 h. Electroporation was performed with 10 µg/mL CD147–GFP plasmid DNA on polarized MDCK cells, and images were obtained by LSCM with 20× objective. Bar: 20 µm. C) TER was measured on MDCK monolayers on filters before, 1 h and 24 h after electroporation (EP) of various plasmids at a concentration of 10–15 µg/mL. No significant differences were observed in TER values before and after electroporation.

Figure 3. Comparison of efficacy of electroporation technique versus cationic lipid reagents Fully polarized MDCK cells were electroporated with 10 $\mu\text{g/mL}$ of CD147–GFP or transfected with lipid reagents, Lipofectamine and Effectene. After overnight incubation, filters were fixed and imaged. A) Entire 12-mm trans-well filters were scanned to have an overview of the transfection in the epithelial monolayer. Bar: 1 mm. B) A magnified single field (white box from A) from the entire 12-mm transwell filter is shown. Bar: 100 μm . C) Individual images were acquired with a high-sensitivity camera for quantification purposes. Total GFP fluorescence from 10 positive fields were averaged and normalized to the number of nuclei as measured by DAPI fluorescence. Arbitrary fluorescence units (GFP/DAPI) obtained with different transfection methods are plotted in the graph.

Figure 4. Electroporation of apical and basolateral markers in polarized MDCK cells

Laser scanning confocal microscope analysis reveals appropriate targeting of the apical markers p75-GFP and GPI-YFP (A), the basolateral markers CD147-GFP and NCAM-GFP (B) and the TGN marker ST-RFP, which localizes to the perinuclear region (C). Polarized MDCK cells were electroporated with 10 $\mu\text{g/mL}$ of all above-mentioned plasmid DNA. Cells were immunostained with ZO-1 (red or green) to assess the integrity of the monolayer. Nuclei were stained with DAPI (blue). Shown are the x-y and x-z projections of the MDCK monolayers. Bar: 10 μm . Western blot analysis of equal amount of total lysates obtained after electroporating nine 12-mm transwell filters with p75-GFP plasmid (D).

Figure 5. Effective electroporation in polarized ARPE-19 cells and successful introduction of antibodies in the polarized MDCK cells

Efficient transfection was feasible in 6-week-old ARPE-19 cells grown on laminin-coated filters. A) An image taken with 20 $\times$ objective shows the overview of the transfection efficiency of CD147-GFP plasmid DNA in polarized ARPE-19 cells. Bar: 20 μ m. A higher magnification (63 $\times$) reveals appropriate apical polarity of CD147-GFP (x - y and x - z projection) B) ARPE-19 cells were immunostained with the endogenous apical marker MCT1 (red). Bar: 10 μ m. C) Proteins can also be introduced in epithelial monolayers, as shown by the uptake of goat immunoglobulin G (IgG)-Alexa 488.

Table 1

Determination of transfection efficiency in MDCK cells electroporated with CD147–GFP plasmid DNA after 24 h

Cell line	Plasmid concentration (µg/mL)	Number of GFP-positive cells/total number of cells (DAPI) counted in the fields underneath the electrode	Transfection efficiency (%) (mean ± SD)
MDCK	5	—	0
	10	92/380	24.51 ± 5.11
	30	203/412	49.69 ± 12.02