Video Article

High efficiency, Site-specific Transfection of Adherent Cells with siRNA Using Microelectrode Arrays (MEA)

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Abstract

The discovery of RNAi pathway in eukaryotes and the subsequent development of RNAi agents, such as siRNA and shRNA, have achieved a potent method for silencing specific genes[1-8] for functional genomics and therapeutics. A major challenge involved in RNAi based studies is the delivery of RNAi agents to targeted cells. Traditional non-viral delivery techniques, such as bulk electroporation and chemical transfection methods often lack the necessary spatial control over delivery and afford poor transfection efficiencies[9-12]. Recent advances in chemical transfection methods such as cationic lipids, cationic polymers and nanoparticles have resulted in highly enhanced transfection efficiencies[13]. However, these techniques still fail to offer precise spatial control over delivery that can immensely benefit miniaturized high-throughput technologies, single cell studies and investigation of cell-cell interactions.

Recent technological advances in gene delivery have enabled high-throughput transfection of adherent cells[14-23], a majority of which use microscale electroporation. Microscale electroporation offers precise spatio-temporal control over delivery (up to single cells) and has been shown to achieve high efficiencies[19, 24-26]. Additionally, electroporation based approaches do not require a prolonged period of incubation (typically 4 hours) with siRNA and DNA complexes as necessary in chemical based transfection methods and lead to direct entry of naked siRNA and DNA molecules into the cell cytoplasm. As a consequence gene expression can be achieved as early as six hours after transfection[27]. Our lab has previously demonstrated the use of microelectrode arrays (MEA) for site-specific transfection in adherent mammalian cell cultures[17-19]. In the MEA based approach, delivery of genetic payload is achieved via localized micro-scale electroporation of cells. An application of electric pulse to selected electrodes generates local electric field that leads to electroporation of cells present in the region of the stimulated electrodes. The independent control of the micro-electrodes provides spatial and temporal control over transfection and also enables multiple transfection based experiments to be performed on the same culture increasing the experimental throughput and reducing culture-to-culture variability.

Here we describe the experimental setup and the protocol for targeted transfection of adherent HeLa cells with a fluorescently tagged scrambled sequence siRNA using electroporation. The same protocol can also be used for transfection of plasmid vectors. Additionally, the protocol described here can be easily extended to a variety of mammalian cell lines with minor modifications. Commercial availability of MEAs with both pre-defined and custom electrode patterns make this technique accessible to most research labs with basic cell culture equipment.

Video Link

The video component of this article can be found at https://www.jove.com/video/4415/

Protocol

1. MEA Preparation

1. MEAs for use in electroporation experiments can either be fabricated using standard photolithography technique as described previously[18] or purchased directly from vendors of MEA manufacturers such as Multi Channel Systems (http:/www.multichannelsystems.com/) and Alpha MED Scientific, Inc. (http://www.MED64.com).The MEAs used in our experiments were fabricated in-house at the clean room facility in Arizona State University, which is administered by the Center for Solid State Electronics Research (CSSER). The MEAs used in our experiments had 32 indium tin oxide electrodes in an 8 by 4 array with electrode diameter sizes from 50-200 μm within a 1 cm inner diameter glass well. The glass well was bonded on the MEA using polydimethylsiloxane (PDMS).
2. Sterilize the MEA in an autoclave prior to cell seeding. Other methods of sterilization such as a soaking in 70% ethanol, UV treatment and hot water treatment as described elsewhere can also be used as long they do not compromise the integrity of the MEA.
3. Transfer the autoclaved MEA to a laminar flow hood and place it in a standard sterile polystyrene Petri dish. Prior to seeding cells on the MEA, sterilize the laminar flow hood by turning on the UV light for 20 min. If the MEA is sensitive to UV light cover the Petri dish containing the MEA with sterile aluminum foil while the flow hood is under UV illumination.

2. Seeding Cells on the MEA

1. Harvest cells from a running cell line of adherent mammalian cells using Trypsin/EDTA and raise them to a concentration of 100-200 cells/µl in 1 ml cell media for plating. In our experiments we have been successful in culturing NIH 3T3 fibroblasts cell line, HeLa cell line and primary cortical and hippocampal neurons on the MEA.
2. Place a 20-30 µl drop of media with cells on the MEA. Transfer the Petri dish with the MEA to the incubator. It is important to ensure that the cell drop size is large enough to cover all the electrodes on the MEA.
3. 2 hr after seeding the cells transfer the Petri dish with the MEA from the incubator to laminar flow hood and gently add 400-500 µl of pre warmed media (37 °C) to the MEA well. Check under the microscope to ensure that cells are still attached and evenly spread on the MEA surface. Transfer the MEA back to the incubator. The 2 hr wait period before the addition of media ensures enough time for the cells to adhere with the MEA surface. Addition of cell media too soon after seeding the cells can wash away the cells. If cells do not adhere well on the MEA, its surface can be treated with cell attachment factors such aspolylysine, fibronectin, and laminin to improve cell adhesion. In our HeLa cell experiments we typically treat the MEA surface with fibronectin (10 µg/ml) for 1-2 hr.
4. 8-12 hr after seeding the cells, perform 1-2 washes with PBS to remove any dead cells. Cell medium can replaced every 24-48 hr as needed. In a typical experiment, the cells are usually ready for transfection 24-48 hr after seeding. To maintain consistency in electroporation efficiency it is recommended to wait until the culture is at least 80% confluent before electroporation.

3. Site-specific Transfection of siRNA

1. Prepare nucleic acid solution. For siRNA transfection, prepare a 1-2 µM siRNA electroporation solution in ice cold electroporation buffer to attain a final volume of 200-400 µl. In this video article we demonstrate the transfection of HeLa cells with Alexa 488 conjugated scrambled sequence of siRNA.
2. Transfer the MEA with cells from the incubator to sterile laminar flow hood. Remove the cell media from the MEA and perform a wash with PBS. Thereafter, add 200-400 µl of the ice cold 1 µM siRNA electroporation solution to the well.
3. Apply anodic square electric pulses to the targeted electrodes (Refer to step 4 for determining optimal parameters of the electric pulse). In our experiments we used a Pragmatic Instruments 2414A waveform generator to generate electric pulses and a dual-inline-package (DIP) 16 pin clip to make contact with the MEA bond pads. The waveform generator can be connected to the DIP clip via a demultiplexor printed circuit board to provide selectivity of individual electrodes. For the reference electrode, place a steel cathode in the cell media. While lowering the steel reference electrode in the media it is extremely important to ensure that it does not make contact with the MEA surface. Upon contact, it can cause damage to the cells and the MEA. It is recommended to lower the reference electrode to a position of 1 mm above the cells. A spacer with a height of 1 mm can be placed in the well to assist in placement of the reference electrode. An alternate approach to using an external reference electrode is to use neighboring electrodes as cathode-anode pairs.
4. Immediately after applying the electric pulses to the targeted electrodes on the MEA, transfer the MEA back to the incubator. After an incubation period of 5 min, gently replace 75% of the electroporation buffer with pre-warmed (37 °C) cell media. Later, fluorescent imaging can be done to confirm the delivery of fluorescently tagged siRNA to the cells.

4. Electroporation Parameter Optimization

1. To determine the optimal electric pulse parameters for electroporation, design electroporation experiments for a range of values of pulse amplitude, pulse duration, and number of pulses. In our experience with HeLa cells, we observed that a single voltage pulse of amplitude from 3 V to 9 V, and duration from 1 msec to 10 msec for an electrode size of 100 µm led to successful electroporation with minimal cell death. It is our recommendation that one parameter be varied at a time and others are kept constant. To quantitate the electroporation efficiency for each of the pulse parameters, we use propidium iodide (PI), a cell impermeant dye that stains nucleic material, and live assay based methodology, as described in steps 4.2-4.5.
2. Establish a cell culture on the MEA following the directions outlined in steps 1 and 2.
3. Prior to electroporation prepare a 300-500 µl of 30 µg/ml PI solution in ice cold electroporation buffer and 400 µl of 4 µM calcien AM solution in PBS.
4. Transfer the MEA from the incubator to the laminar flow hood. Remove the cell media from the MEA and perform a wash with PBS. Thereafter, add 300-500 µl of the ice cold electroporation buffer with PI.
5. Apply electric pulses with desired pulse amplitude and duration to the targeted electrodes (The equipment used in our experiments for generating and applying the electric pulse to the MEA is described in step 3.3). For the reference electrode either lower a steel electrode into the electroporation buffer in the MEA well or use the neighboring electrode as reference. By selectively applying pulses of varying amplitudes or durations to different electrodes multiple experiments can be performed on the same device. For example, on a 32 electrode MEA, 8 experiments can be performed by splitting the 32 electrodes in 8 groups, each consisting of 4 electrodes. Each of the 8 groups can then be stimulated by a single rectangular voltage pulse of duration 5 msec with amplitudes varying in 3 V-6 V range in 0.5 V increments and one group can be used as a control.
6. Immediately after applying the voltage pulses transfer the MEA to the incubator for 5 min. Afterwards, gently remove 75% of the electroporation buffer from the MEA well and replace it with warm cell media. Transfer the MEA back to the incubator.
7. After an incubation period of 2-4 hr replace the cell media in the MEA well with 300-500 µl of 4 µM Calcein AM solution in PBS. Transfer the MEA back to incubator for another 30 min.
8. Following the incubation of the cells with Calcein AM, replace the Calcein AM solution in the MEA well with PBS after two washes with PBS. Obtain fluorescent images of cells using with an optical microscope with filters for PI (Excitation/Emission - 535 nm/617 nm) and Calcein AM (Excitation/Emission - 490 nm/520 nm). The uptake of PI, due to electroporation, causes cells to fluoresce red and the Calcein AM causes cells that are alive to fluoresce green. Three possible scenarios that can be observed are 1) cell electroporated and alive (will fluoresce both red and green), 2) Cells alive but not electroporated (will fluoresce just green) and 3) Cells dead due to electroporation (will fluoresce just red). The cell viability was calculated as a percent of the total number of cells alive on the electrode and the electroporation efficiency was calculated as percent of the total number of cells loaded with PI/siRNA.

5. Representative Results

Figure 1 shows the site-specific loading of HeLA cells with PI. It can be observed that only cells on the electrode demonstrate an uptake of PI (**Figure 1B**). A live assay performed post electroporation was used to assess the viability of cells (**Figure 1A**). The electroporation efficiency and cell viability for the example shown in **Figure 1** are 81.8 % and 96.1 % respectively. High cell viability and electroporation efficiency can be achieved by optimizing the electroporation pulse parameters. Site-specific transfection of HeLA cells with fluorescently tagged siRNA for an optimized electroporation pulse (8 V, 1 msec) is shown in **Figure 2**. The electroporation efficiency for the siRNA at 8 V, 1 msec was found to be 74.4 ± 16.0 % and cell viability was found to be 87.9 ± 8.4 % (all data represented as mean ± standard deviation, N=5).

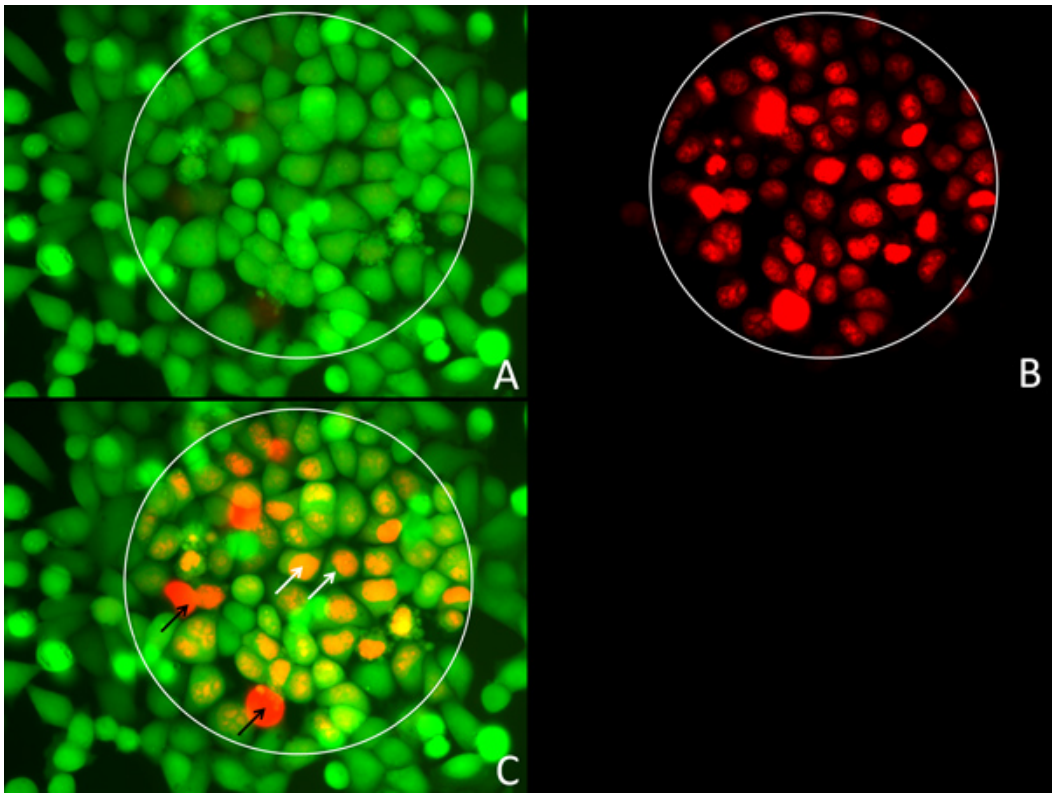

Figure 1. Site-specific delivery of PI in HeLa cells on a 200 µm electrode (6 V, 5 msec). A) Calcien based live assay performed 2 hr post electroporation. Alive cells are indicated by green fluorescence. B) Red fluorescence demonstrates the uptake of PI by cells on the electrode. C) A superimposed image of A and B. The white arrows indicate cells that are electroporated and alive. The black arrows indicate cells that are dead. The white circle in A, B and C indicates the outline of the electrode.

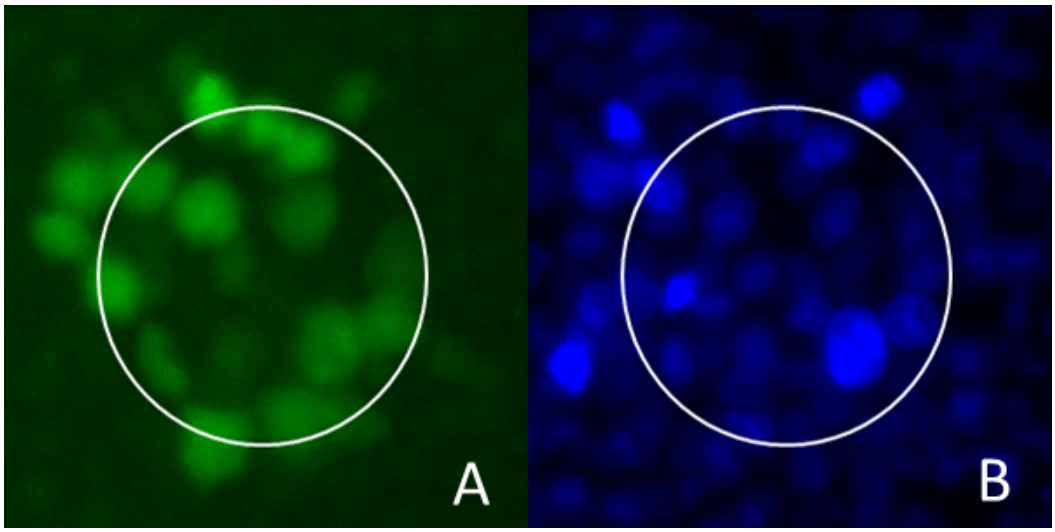

Figure 2. Transfection of HeLa cells with fluorescently tagged scramble sequence of siRNA. A) Image of HeLa cells on a 100 µm electrode transfected with Alexa 488 tagged siRNA (8 V, 1 msec). B) Image of cell nuclei stained with DAPI. The white circle marks the edge of the electrode.

Discussion

In this video article we demonstrate the use of MEA for site-specific transfection of HeLa cells with scrambled sequence of siRNA. One of the advantages of this technology is its applicability to different cell lines including primary cell lines. Our lab has previously demonstrated the use of this technology for site-specific transfection of primary hippocampal neuronal culture from E18 day old rat and NIH-3T3 cells with scrambled siRNA sequences and GFP plasmid[18, 19]. We have also had success in delivering functional siRNA against an endogenous target in HeLa cells (unpublished data). A typical commercially available MEA is compatible with optical imaging (upright microscope), enabling quantitative assessment of functional impact of gene silencing, using fluorescent immunostaining techniques. Additionally, the microelectrodes in MEA can be used to monitor electrical activity of cells, such as primary neurons, in response to genetic perturbation. The unique nature of MEA enables simultaneous multiple experiments on a single cell culture, thereby increasing the experimental throughput. Currently a major limitation with the MEA based system is the large amount of siRNA needed for transfection (400 µl of 1µM siRNA), which is high compared to conventional chemical transfection based approaches and thus limits the cost effectiveness of the system. This can partly be overcome by incorporating mircofluidics with the MEA based system to decrease the total volume of siRNA electroporation solution to less than a few microliters. Additionally, the microfluidics can enable serial delivery of different siRNA molecules; thereby greatly enhancing the throughput of the MEA based technology. Alternatively, siRNA molecules can be spotted on the microelectrodes prior to cell seeding and then the siRNA molecules can be transfected into cells by reverse electroporation[15, 23]. Further development of this technology offers a novel high-throughput, efficient and low cost method for gene manipulation studies.

Disclosures

No conflicts of interest declared.

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