Biomaterials 67 (2015) 225–231

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Spatio-temporally controlled transfection by quantitative injection into a single cell

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ARTICLE INFO

Article history:
Received 1 July 2015
Accepted 11 July 2015
Available online 21 July 2015

Keywords:
Quantitative
Single cell
Transfection
Spatio-temporal

ABSTRACT

Transfection-based cellular control has been widely used in biology; however, conventional transfection methods cannot control spatio-temporal differences in gene expression or the quantity of delivered materials such as external DNA or RNA. Here, we present a non-viral and spatio-temporally controlled transfection technique of a quantitative injection into a single cell. DNA was quantitatively injected into a single cell at a desired location and time, and the optimal gene delivery and expression conditions were determined based on the amount of the delivered DNA and the transfection efficacy. Interestingly, an injection of 1500 DNAs produced an about average 30% gene expression efficiency, which was the optimal condition, and gene expression was sustained for more than 14 days. In a single cell, fluorescent intensity and polymerase chain reaction (PCR) results were compared for the quantity of gene expression. The high coincidence of both results suggests that the fluorescence intensity can reveal gene expression level which was investigated by PCR. In addition, 3 multiple DNA genes were successfully expressed in a single cell with different ratio. Overall, these results demonstrate that spatio-temporally controlled transfection by quantitative transfection is a useful technique for regulating gene expression in a single cell, which suggests that this technique may be used for stem cell research, including the creation of induced pluripotent stem (iPS) cells.

1. Introduction

Transfection has been widely used as an investigative tool to study gene function and gene expression in eukaryotic cells [1–4]. For example, transduction by viral vectors has been shown to be highly efficient in creating cellular changes, and lipofection, also known as lipid-mediated DNA transfection, can be safely attained without utilizing mutagenesis in the transfection procedure [5,6]. Although cellular changes occur in individual cells, determining and adjusting the quantity of the delivered external materials in each cell for the transfection still remains challenging [7].

Here, we report a spatio-temporally controlled single-cell transfection method using quantitative injection. In general, viral transduction materials are dispersed into the entire cell media and randomly adhere to each cell (Fig. 1a). By contrast, we directly delivered a quantitative volume of non-viral transfection materials into a single cell using an injection system with a 500 nm inner diameter tip (Fig. 1b) [8–11]. By controlling the injection pressure and duration, the volume delivered into a single cell was calculated using Poiseuille's law [12–16]. Finally, the number of delivered genes was also calculated based on the concentration and the injected volume of the DNA solution. As shown in Fig. 1b, precise amounts of external DNA were directly delivered into the nucleus. Therefore, we determined the gene expression efficacy, strength, and sustained rate as a function of the delivered DNA quantity. In addition, the transfected cells were free from mutagenesis and cytotoxicity complications because viral- and lipid-mediated vectors were not used [17–20]. This technique may be used in future clinical applications.

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http://dx.doi.org/10.1016/j.biomaterials.2015.07.024
0142-9612/

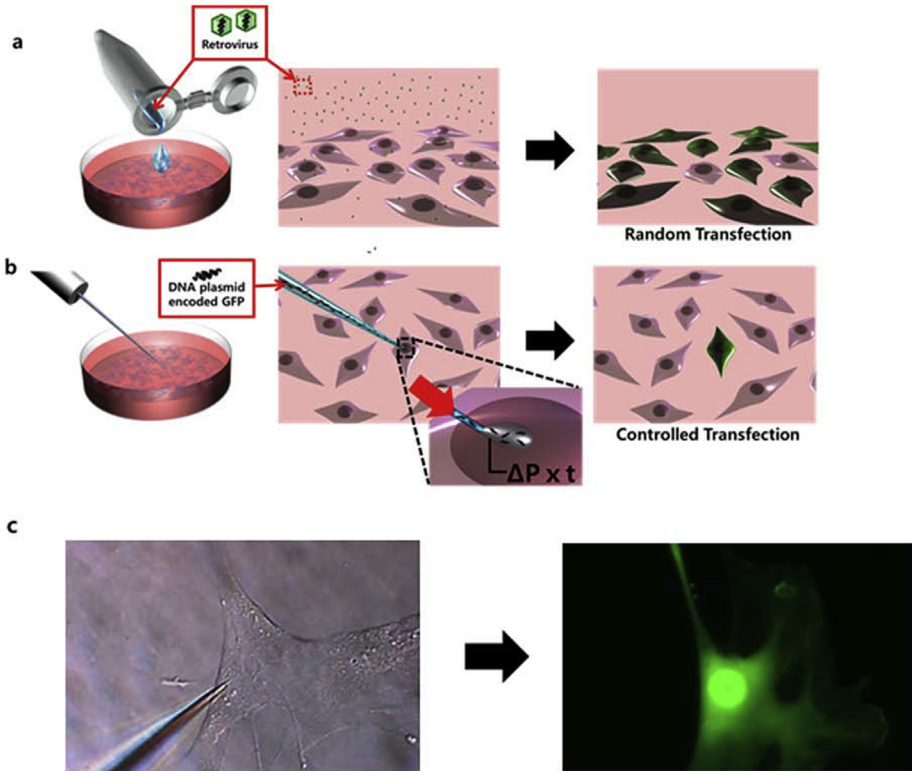

Fig. 1. Schematic diagrams and representative results from the transfection. (a) A conventional transduction method using a retrovirus (left). When transduction materials were dispersed into the entire cell media, they randomly adhered to each cell (middle), and the transduction was uncontrollable within a single cell unit (right). (b) Non-viral transfection material was delivered by quantitative injection (left). This method produced a fine injection volume that was delivered into a single cell by the regulating injection pressure and injection time (t); ΔP was calculated as the difference between the injection pressure from the injector and the intracellular pressure (middle). This method produced a controlled transfection into a single cell (right). (c) Optical image (left) showing the experimental process and a fluorescent image (right) obtained 24 h following a DNA(pcDNA3.3_eGFP) injection.

2. Materials and methods

2.1. DNA preparation for single cell injections

Mammalian expression fluorescent gene plasmids were purchased from Addgene (Cambridge, MA, USA; pcDNA3.3 eGFP, pCSCMV:Td-tomato, pEBFP2-Nuc). The plasmids containing *Escherichia coli* were cultured in 100 ml LB media supplemented with antibiotics overnight at 37 °C and 300 rpm in a shaking incubator for 12–16 h. The grown *E. coli* cell cultures were harvested by centrifugation at 5000 × g for 10 min, and the supernatant was discarded. The NucleoBond® Xtra Midi plasmid DNA purification kit (Macherey–Nagel, Düren, Germany) was used for DNA preparation according to the manufacturer's instructions. After a final wash of the plasmid DNA pellet, the DNA pellet was dissolved in 50 μl of DNase-free/RNase-free water, and the plasmid yield was determined using a Nanodrop2000 (Thermo Scientific HyClone, Logan, UT, USA).

2.2. Cell isolation and culture

Mouse embryonic fibroblast (MEF) cells were used for the transfection experiment. To isolate the MEF cells, skin tissue fragments from 13-day mouse embryos were collected and trypsinized for 15 min and pipetted vigorously to separate the tissue into single cells. The trypsinized cells were filtered with 100 μm and 40 μm cell strainers (BD Biosciences, San Jose, CA, USA) and centrifuged to create cell pellets. After the supernatants were removed, the cell pellets were resuspended with fibroblast culture media containing Dulbecco's modified Eagle medium (DMEM, Thermo Scientific HyClone) with 10% FBS (Thermo Scientific HyClone), 2 mM L-glutamine (GIBCO, Carlsbad, CA, USA), 0.1 mM MEM NEAA (GIBCO) and penicillin/streptomycin (GIBCO). The MEF cells were cultured on a cell culture plate at 37 °C with 5% CO_2 for expansion.

2.3. Single cell injections and fluorescence imaging

For the transfection with Femtotips®II (Eppendorf, Hamburg, Germany), 1 × 10^5 MEF cells were plated in a 35 mm culture dish (BD Biosciences, San Jose, CA) the day prior to injection. Immediately prior to injection, the fibroblasts were washed with PBS (GIBCO) and fibroblast culture media. The fibroblasts were injected with Femtotips®II that were loaded with a small molecule (DAPI, 1:1000) or a fluorescent protein plasmid (GFP only or GFP/Td-tomato/BFP mixture) using the FemtoJet® microinjection instrument (Eppendorf, Hamburg, Germany) and a micromanipulator (MP-225, Sutter instrument Co., Novato, CA, USA). The plasmid injection volume varied and was controlled by the injection pressure (1200–1800 hPa) and injection time (0.3 s-1.2 s). The plasmid DNA was injected into the nucleus of the fibroblast, and the injected cells were washed with PBS a minimum of 2 times and then cultured at 37 °C under 5% CO_2. Beginning 1 day after the injection, the fluorescent gene expression levels of the injected cells were observed using fluorescence microscopy, and the efficacy and fluorescence intensity were recorded every day for 7 days.

2.4. Single cell real time PCR

Total RNA was prepared using Cell Lysis Buffer (Signosis, Santa Clara, CA, USA) following the manufacturer's instructions. Three