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Automatic positioning of a microinjector in mouse ES cells and rice protoplasts

Hideaki Matsuoka [a,b,*], Soichiro Shimoda [a], Yoshiyuki Miwa [c], Mikako Saito [a,b]

[a] Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan
[b] CREST (Core Research for Evolutional Science and Technology), Japan Science and Technology Agency, Honcho 4-1-8, Kawaguchi, Saitama 332-0012, Japan
[c] Faculty of Science and Engineering, Waseda University, 3-4-1 Ookubo, Shinjuku-ku, Tokyo 169-8555, Japan

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Abstract

A 2-channel (2C) microinjector was prepared by pulling a glass capillary with a θ-shaped cross section. One channel was used as a potential measuring electrode (MeaE) and the other was used as an electrophoretic introduction electrode (IntE). The 2C microinjector was propelled by an oil pressure manipulator driven by a pulse motor, while the MeaE output was recorded continuously. When the 2C microinjector penetrated the cell membrane of a mouse ES cell or a rice protoplast, the output potential changed sharply. The differential of this potential change was used as a stop signal for the pulse motor. Thus, the microinjector was correctly positioned in the cell without losing cell viability. Its success rate was 73% and 84% for ES cells and rice protoplasts, respectively. After the positioning of the microinjector in the cell, Lucifer yellow (LY) was introduced via IntE. Under these conditions, the rate of viable cells was 16% and 62% for ES cells and rice protoplasts, respectively.

Keywords: Microinjector; Automatic positioning; Intracellular potential; ES cell; Rice protoplast

1. Introduction

An intense need for the direct introduction of genes, expression products or associated bioactive compounds into target cells has arisen in order to analyze protein–protein interaction [1], intracellular [2,3], or intercellular [4–6], signaling, intracellular localization or trafficking [7,8], and gene targeting efficiency [9]. Thus, microinjection has now attracted substantial attention because of its potential usefulness. However, microinjection requires a high degree of expertise and is very time consuming. Therefore, it is not considered practical for the rapid treatment of large number of cells. Recently, difficulties have partially been overcome by the development of a unique machine, the single-cell manipulation supporting robot (SMSR) [10]. SMSR has enabled high-throughput microinjection. Moreover, SMSR was found to be useful for training purposes.

In spite of its usefulness, there remains a need for further improvement towards fully automatic microinjection. To accomplish this final goal, it is essential to develop a controller that can stop the microinjector at the proper position in the cell. Formerly various plates with multiple microwells or multiple pores were devised [11–18], and ideas of automatic microinjection using these plates were proposed [13,14,17]. However no successful demonstration was reported. In these cases, the microinjector was propelled to the XY-address of the center of the well or the pore. Consequently the microinjector penetrated the center of the cell and this killed most cells, except those of 50 μm or more in diameter or exceptionally resilient cells [19,20].

According to the suggestions of microinjection experts (personal communications), it is important to make the penetration depth of the microinjector as shallow as possible. Therefore, it is necessary to stop the microinjector immediately when the tip penetrates the cell membrane. However, it is difficult to determine the stop position by the XY-address based on the well or pore position, because cell diameters vary from cell to cell. Moreover, cell diameter changes due to cell deformation

* Corresponding author. Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan. Tel.: +81 42 388 7029; fax: +81 42 387 1503.
E-mail address: bio-func@cc.tuat.ac.jp (H. Matsuoka).

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when the microinjector presses and then penetrates the cell membrane. Therefore it is necessary to use another stop signal that does not depend on these dimensional factors.

When a microelectrode is propelled towards the cell membrane at a right angle to the cell membrane, a steady potential is obtained. This potential will change sharply when the microelectrode contacts with and then penetrates the cell membrane. Subsequently, the potential again becomes steady, and this is designated as the intracellular potential. Such a potential change may be used as a stop signal that is independent of cell diameter and cell deformation.

This paper describes a novel method of automatic microinjector positioning. We have devised an electric circuit that can sensitively measure the intracellular potential without serious electric noise. Thus, the measured potential change is modified to generate an effective stop signal. The performance of the present method was then tested with mouse ES cells and rice protoplasts.

2. Materials and methods

2.1. Preparation of 2-channel microinjector

A glass capillary with a θ-shaped cross section (Fig. 1) (outer diameter, 1 mm) was heated and pulled to make the tip diameter smaller than 1 μm using a laser puller (Model 2000, Sutter Instrument Co., Novato, CA). One channel of the capillary was filled with a solution of 0.5 M KCl. Ag/AgCl wire was then inserted into the capillary to produce a potential measuring electrode (MeaE) (Fig. 1). The reference electrode for MeaE was a commercially available Ag/AgCl electrode (RE). The other channel was filled with a solution containing 0.5 M KCl and 1 mM Lucifer yellow (LY). Pt wire was inserted into the capillary to produce a dye-introducing electrode (IntE). The counter electrode (CE) for IntE was prepared by filling a glass tube with 3% agar gel containing 0.5 M KCl.

2.2. Apparatus construction

The MeaE and RE were connected to an electrometer (Fig. 1). The output of the electrometer was connected to a signal converter and a programmable controller for a pulse motor driver (SC/MD). From the output, digital pulses were generated at 25 Hz and supplied to a pulse motor connected directly to an oil pressure manipulator (OM) (MO-22, Narishige Kagaku, Tokyo). The clockwise rotation of the OM propels the microinjector. The minimum step of the microinjector is 0.25 μm and the propelling speed is 6.25 μm s^{-1}.

The IntE and CE were connected to a pulse generator (PG) via an electric isolator (Iso). Rectangular analog pulses were generated by PG, and intensified and supplied to the IntE such that the voltage of IntE was −20 V vs. CE (pulse width: 10–50 ms; pulse number: 1–5 pulses with 1 s interval). These conditions were determined so that the amount of LY expelled from the microinjector was enough for direct observation with a fluorescent microscope.

2.3. Conversion of intracellular potential signal

The output of MeaE vs. RE was amplified and input into a low-pass filter circuit. This signal was further converted to its differential and transferred to a comparator circuit. The intracellular potential or its differential was recorded on a pen recorder (Rec). Circuit parameters for the time constant and amplification factor were adjusted for mouse ES cells and rice protoplasts, respectively.

2.4. Mouse ES cells culture

Feeder free mouse ES cells were provided by H. Niwa (Center for Developmental Biology, RIKEN, Kobe) and were cultured at 37 °C in the absence of feeder cells in Glasgow

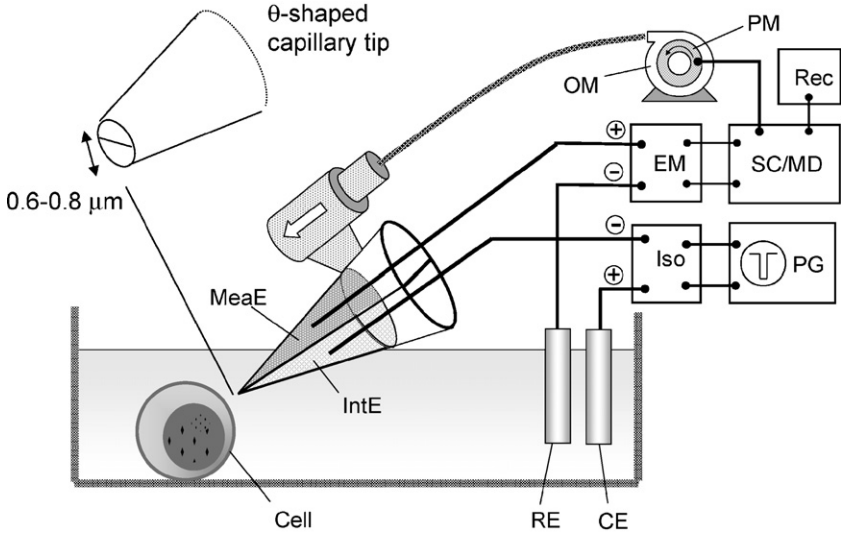

Fig. 1. Schematic diagram of an automatic microinjector positioning system. MeaE: potential measuring electrode, IntE: dye introduction electrode, RE: reference electrode for MeaE, CE: counter electrode for IntE, Iso: isolator, PG: pulse generator, EM: electrometer, SC/MD: signal converter and motor driver, PM: pulse motor, OM: oil pressure manipulator, Rec: recorder.