Electroporation of Smooth Muscle Cells Using the Gene Pulser MXcell™ Electroporation System

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Introduction

Smooth muscle cells (SMCs) lie at the center of a number of pathologies throughout the body, including vascular proliferative diseases such as atherosclerosis, restenosis, vein graft stenosis, and asthma. In order to study SMC biology in general and the mechanisms of pathogenesis of these diseases in particular, molecular manipulation of these cells is necessary. However, transfection of these cells, especially primary isolates from human origin, has been difficult. In this report, we have used the Gene Pulser MXcell electroporation system to rapidly optimize experimental conditions for transfection of several SMC types. The benefit of the Gene Pulser MXcell electroporation system is that it allows the user to optimize transfection protocols to multiple cell types at the same time, using a 96-well format. Using this system, we optimized transfection of three different human SMC primary isolates: aortic intimal SMC, pulmonary artery SMC, and bronchial SMC.

Methods

Primary isolated human aortic intimal SMCs and human pulmonary artery SMCs were cultured in GIBCO Dulbecco's Modified Eagle Medium (DMEM, Invitrogen Corporation) supplemented with 10% fetal bovine serum (FBS). Bronchial SMCs were cultured in Smooth Muscle Basal Medium (SmBM, Lonza Group Ltd) at 37°C. Cells were passaged 1 day prior to electroporation, so at the time of electroporation the cells were approximately 80–90% confluent and actively dividing. Cells were washed with phosphate buffered saline (PBS) and trypsinized. All three cell types were then centrifuged and resuspended in serum-free DMEM at a cell density of 2 x 10^6. A reporter plasmid encoding green fluorescent protein (GFP) driven by a CMV promoter (pEGFP-N1, Clontech) was added to each cell suspension at a concentration of 20 µg/ml. Aliquots of 200 µl were then transferred into each of the wells of a 96-well electroporation plate and electroporated with the Gene Pulser MXcell system (Bio-Rad Laboratories, Inc.). After electroporation, the cells were transferred to 24-well culture plates, each well containing 2 ml of DMEM with 10% FBS or SmBM, and grown for 24 hr at 37°C. At 24 hr, cells were visualized by epifluorescence microscopy (Leica DMI 6000B, Leica Microsystems) and imaged using Volocity imaging software (PerkinElmer, Inc.) to detect GFP in electroporated cells. After imaging, cells were washed with PBS, trypsinized, and counted using trypan blue exclusion to determine viability. Samples were then spun, fixed in ice cold 1% formaldehyde for 15 min, and resuspended in PBS in order to run them on a BD FACSCalibur flow cytometer (BD Biosciences) to detect GFP-expressing cells.

Results and Discussion

In order to optimize electroporation parameters for different types of SMCs, the Gene Pulser MXcell system was used to simultaneously test several parameters. Cell viability and transfection efficiency were recorded for each sample.

Effect of Voltage

Voltage conditions between 150 and 450 V were applied to the three types of SMC while capacitance was held constant at 350 µF. All three SMC types had declined survival with increased voltage (Figure 1). Of the three cell types, SMCs from the pulmonary artery showed the greatest viability at all field strengths, with the aortic intimal SMCs showing intermediate viability and the bronchial SMCs being the most sensitive to increasing fields. The transfection efficiency of the cell lines varied with the increasing voltage and there was generally a trade-off with cell survival vs. DNA delivery (Figure 1). For both aortic intimal and pulmonary artery SMCs, optimal transfection was obtained with an exponential waveform at 450 V, 350 µF, and 1,000 Ω, whereas bronchial SMCs showed greatest transfection at 350 V. The optimal voltage to apply will be dependent on the application of the cells posttransfection.

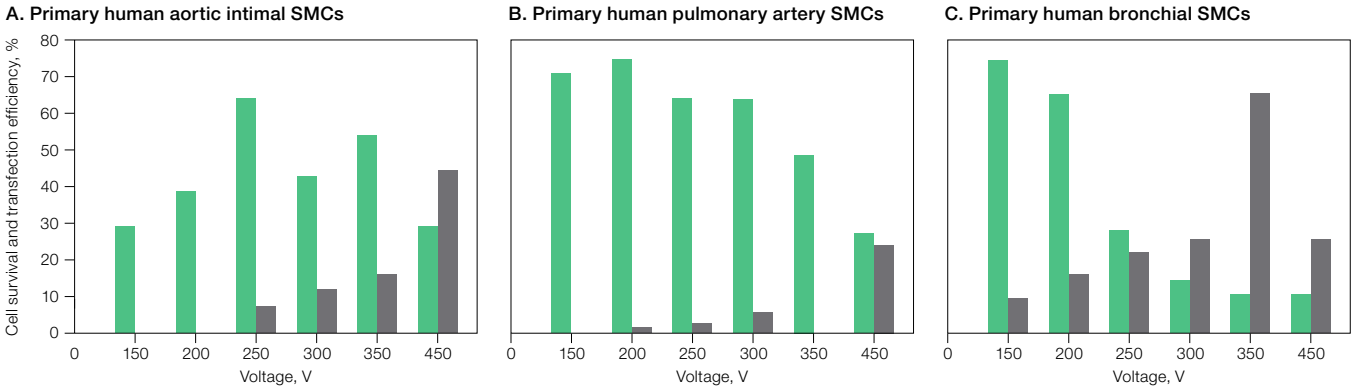

Fig. 1. Effect of voltage on survival and transfection efficiency of primary human SMCs. A, primary human aortic intimal SMCs; **B**, primary human pulmonary artery SMCs; **C**, primary human bronchial SMCs. Cells were exposed to an exponential waveform with 350 µF capacitance, 1,000 Ω resistance, and varying voltage. Both cell survival (■) and transfection efficiency (■) were recorded. GFP-positive cells are reported as percentage of viable cells.

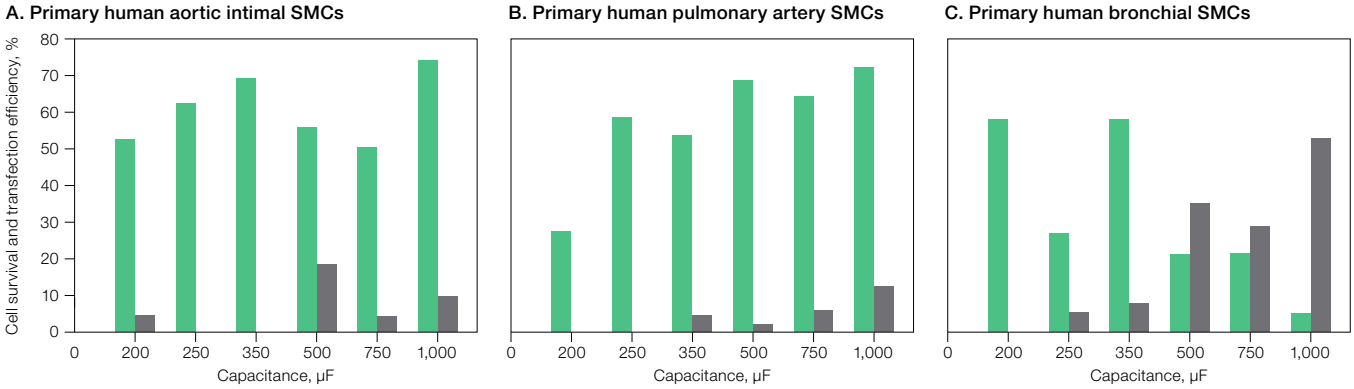

Fig. 2. Effect of capacitance on survival and transfection efficiency of primary human SMCs. A, primary human aortic intimal SMCs; **B**, primary human pulmonary artery SMCs; **C**, primary human bronchial SMCs. Cells were exposed to an exponential waveform with 350 V, 1,000 Ω resistance, and varying capacitance. Both cell survival (■) and transfection efficiency (■) were recorded. GFP-positive cells are reported as percentage of viable cells.

Effect of Capacitance

The capacitance was varied from 200 µF to 1,000 µF with voltage held constant at 350 V (Figure 2). The different cell types varied both in cell survival and in electroporation efficiency; however, the higher capacitance led to a higher transfection rate and had little effect on cell survival in both the pulmonary artery SMCs and aortic intimal SMCs. Higher capacitance settings resulted in poor cell survival in bronchial SMCs; however, high transfection efficiencies could be reached by using a greater number of initial cells.

Exponential vs. Square Waveforms

Both exponential and square waveforms were tested for the three different SMCs. Although square-wave electroporation was capable of transfecting SMCs, the efficiency was far lower than that of the exponential waveforms, so only exponential results are presented.

Conclusions

The Opt mini 96-well/Sqr, Exp preset protocols on the Gene Pulser MXcell electroporation system were used in these experiments to begin to optimize electroporation-mediated transfection into three different primary human SMC types. The ease of the system is such that multiple cell types can be simultaneously tested in one single experiment, limiting time, reagents, and effort. The differential response of these SMCs of different origin to varying voltages and capacitance, in terms of viability and gene expression, highlights the fact that every cell is unique and that electroporation parameters cannot be simply applied from one cell to another.

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