Application Note: InSitu Porator - 001
August 2011

Determining Optimal Electroporation Conditions to Study Gap Junction Communication in Adherent Cardiomyocytes using the InSitu Porator™

Introduction

This application note presents an easy to use optimisation strategy that can significantly improve results, while studying Gap Junction Intercellular Communication (GJIC) in neonatal cardiomyocytes. It details how the optimisation of parameters such as voltage intensity and cell culture density can considerably improve results when using the InSitu Porator™.

Materials & Method

For maximum adhesion of the cardiomyocytes and to generate a confluent monolayer on the glass surface, first coat the slides with collagen. For instructions to make the collagen coating refer to full article.(*) (collagen coating is required only for less adherent cell lines).

To determine optimal cell culture, when using the 8-chamber slides, three densities were tested 15×10^4, $20\times10^4$and 25×10^4 cells / 500 µl growth medium/slide (1,2,3).

To study the effect of the gap junction uncoupler carbenoxolone, the cells were pre-incubated for 30 minutes with 100 µM carbenoxolone.

The electroporation buffer contains: 136mM NaCl, 4mM KCl, 10mM HEPES, 0.5mM NaH_2PO_4, 1mM EGTA, 0.8mM $MgCl_2$, 5mM glucose, and 10% FBS, pH adjusted to 7.4 with NaOH. EGTA is added to the buffer to avoid calcium overload during electroporation.

Due to the modest height of the cardiomyocyte monolayer, use a relatively high Lucifer Yellow (LY) concentration (Add 10 mg /ml buffer and readjust pH to 7.4).
Stain the cells using 200 µl buffer with high grade Lucifer Yellow (LY) per chamber (8-well slide).

Using high grade LY will help avoid impurities which could lead to unspecific fluorescence in the non-electroporated areas.

To determine optimal electroporation intensity the cells were gently electroporated within the range of 10 to 30 volts, as this results in a functional cellular uptake of LY without damaging or stressing the cells (3). Following electroporation, remove all extracellular LY and lightly rinse cells using the same electroporation buffer without the LY. To obtain exact quantification of intercellular spread of LY, carefully remove the plastic chamber and fix cells immediately in 4% formaldehyde in PBS. To analyse live cells, leave the chamber on and view using an inverted microscope. To study comparisons in live cells, images should be taken immediately after electroporation.

Results

The ventricular myocytes were isolated by a method modified from Simpson and Savion (1982) by multiple rounds of trypsin digestions of ventricles from neonatal Wistar rats (Calloe K, *et al.*, 2007) . They can be used for experiments between days 4 and 8 in culture. Three different cell densities (15×10^4, $20\times10^4$and 25×10^4 cells / 500 µl media) were used with the 8-chamber slide. Slides with density 20×10^4 cells/500 µl media, had the most uniformly confluent monolayer, thus this concentration was used for further testing of the system.

For the optimal electroporation intensity, dye uptake at electroporation intensities ranging from 10 to 30 volts (InSitu Porator™ mild electroporation mode) was measured. The degree of gap junctional intercellular coupling after electroporation was calculated as the ratio between the spread of dye and the level of dye uptake in the electroporated cells (Hofgaard JP, *et al.*, 2009).

Cell Projects Ltd
e-mail: info@cellprojects.com - web: www.cellprojects.com

Electroporation intensities ranging from 15 to 30 volts resulted in an equivalent ratio between the uptake and spread of LY as shown in Figure 1, indicating that electroporation in this range is optimal for experiments. No cell damage was observed whilst using the InSitu Porator™ at these electroporation intensities.

Figure 1: Optimal Electroporation Intensity

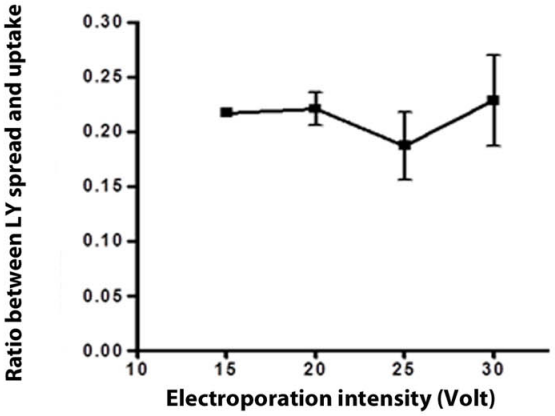
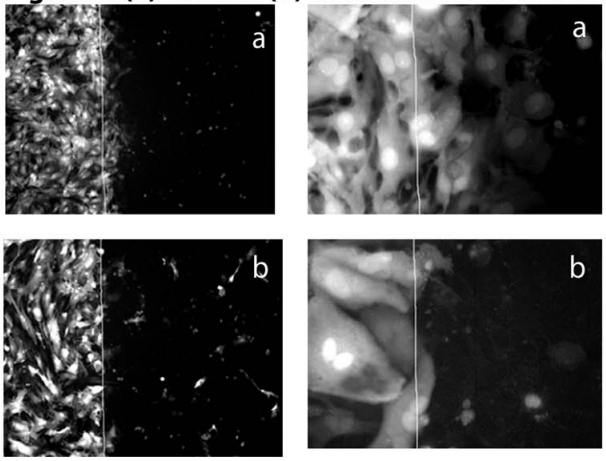


Figure 2 shows fluorescence images of two untreated and two carbenoxolone treated slides after electroporation (10x and 40x magnification). Carbenoxolone reduced the intercellular coupling between the cells (Hofgaard JP, *et al.,* 2008). The white line indicates the border between the conductive (left) and nonconductive (right) surface of the slide. Cells growing on the conductive layer are electroporated and show bright fluorescence. The fluorescence to the right of the line is caused by diffusion of dye through gap junction.

Figure 2: (a) Control (b) Carbenoxolone treated



Conclusion

The findings listed in the method and results form the basis for the practical protocol with optimised conditions for the electroporation of neonatal cardiomyocytes. The results highlight how optimisation of parameters can play a vital role in enhancing electroporation efficiency. In addition, the InSitu Porator™ proved to be an easy to use and powerful tool to study Gap Junction Intercellular Communication.

Notes

1. Do not let the cells dry out at any stage before and after electroporation, as this will result in cell death.

2. Avoid variation in cell preparations e.g. clusters of cells or areas with few cells, as this will result in uneven or unspecific electroporation.

3. When comparison between different slides is needed, maintain a constant degree of cellular confluence and LY volume in the chambers throughout electroporation.

References

Simpson P, Savion S (1982) PMID: 7053872

Calloe K, Nielsen MS, Grunnet M, Schmitt N, Jorgensen NK (2007) PMID: 17442416

Hofgaard JP, Mollerup S, Holstein-RathlouNH and Nielsen MS (2009)PMID: 19535680

Hofgaard JP, Banach K, Mollerup S, Jorgensen HK, Olesen SP, Holstein-Rathlou NH and Nielsen MS (2008) PMID: 18536930

The results were presented by Johannes Hofgaard and Morten Schak Nielsen, Associate Professors at the University of Copenhagen - Faculty of Health Sciences, Department of Biomedical Sciences

(*) To request a copy of the comprehensive article containing material and methods, and results email info@cellprojects.com