

Transfection of Dissociated Hippocampal Neurons in Culture

Cell type:
Rat Hippocampal
neurons (Lonza,
R-Hi-501)

System:
Cellaxess ACE

Simple and reproducible transfection of cryopreserved hippocampal neurons

Direct transfection of differentiated networks

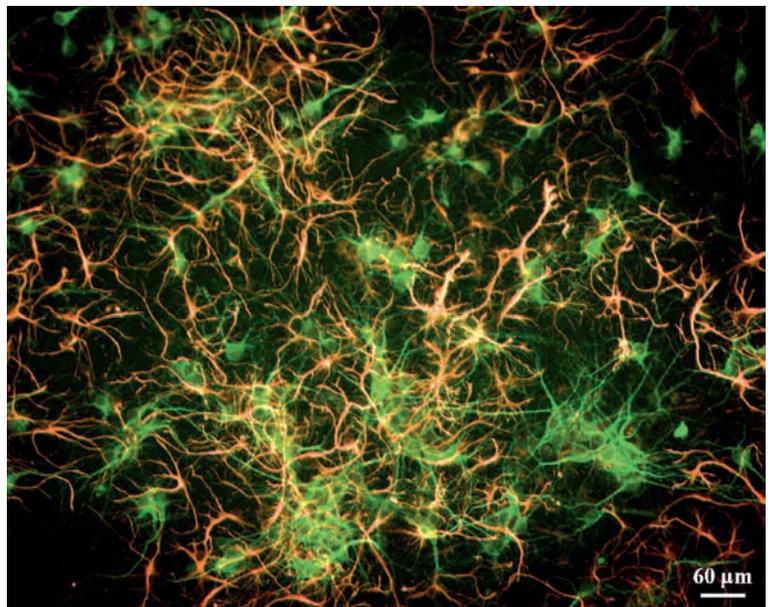
35% transfection efficiency

Introduction

Dissociated hippocampal neurons in culture are considered to be a highly relevant in-vitro model in the study of neuronal development and activity. However, preparation and transfection of hippocampal neurons is highly challenging, hampering the research using this highly relevant cell model. The preparation of hippocampal neurons requires access to laboratory animals and dissecting equipment. Furthermore, the preparation efficiency and quality of the final culture is dependent on the skill of the operator. With regards to transfection of primary hippocampal neurons, non-viral chemical and physical methods such as cationic lipids, biolistic delivery or microinjection typically don't provide a good percentage of healthy cells [1,2]. Viral delivery systems such as adeno-associated or semliki-forest like viruses provides high efficiencies, but the construction of the vectors is time-consuming. Furthermore, the vector itself can provoke an immune response and interfere with metabolic cell processes [3, 4].

Here we present a Cellaxess-based method for transfection of networks of hippocampal neurons in culture prepared from cryopreserved cells. This enables simple, highly efficient and reproducible transfection without significant impact on the cell viability.

Figure 1



Fluorescence micrograph showing the typical ratio of neurons (green) to glial cells (red).

Results

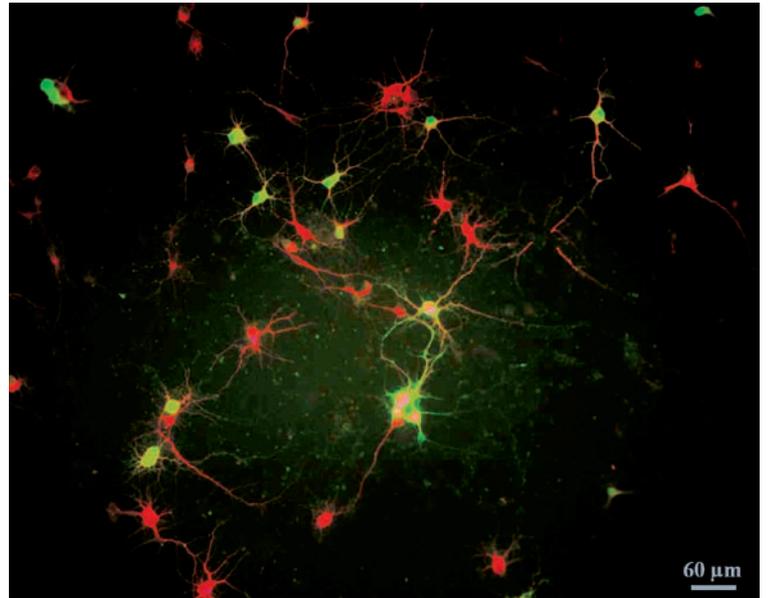
Transfection efficiency and selectivity. Expression of CopGFP fluorescent protein was evaluated at 24, 48 and 72 hours post transfection by fluorescence microscopy. For cell identification cultures were fixed and stained with neuron specific (MAP2) and glial specific (GFAP) antibodies. Figure 1 shows neurons and glial cells stained with MAP2 (green) and GFAP (red) respectively. The transfection was neuron specific with up to 35% transfection efficiency for neurons and less than 5% for glial cells using an optimized protocol for neurons. Figure 2 shows a typical transfected culture at 48 hours. The culture has been stained with antibodies for MAP2 (red) and CopGFP expressing cells are green.

Cell quality. CopGFP expression was stable for at least 72 hours after electroporation, as seen in Figure 3 and 4, acquired 72 hours post transfection. Cells cultured for 12 days developed a highly differentiated network and could be transfected with comparable transfection efficiencies. The CopGFP expression was intense also in the outermost extensions of dendrites and axons and it was straightforward to visualise a high amount of spines on transfected neurons.

Transfection with the Cellaxess ACE system

The plate containing the cells to be transfected was removed from the incubator. The transfection solution containing the pCopGFP vector (Eurogen) at 100-200 ng/ μ l final concentration was pipetted into the ACE capillary by means of a standard p100 micropipette. The dish was positioned under the ACE module, and the capillary was lowered into the dish. After an approximated 15 second wait time, the pre-programmed transfection protocol was executed. The capillary was raised, the dish was temporarily removed, and remaining solution was drained by blowing through the capillary using a p1000 micropipette. After this, the capillary was once again filled with plasmid solution, and the next transfection was carried out. Once the desired number of transfections had been carried out, the dish or plate was returned to the incubator. The optimum transfection protocol consisted of 2 pulses of 1 ms pulse length with an interval of 15 seconds and amplitude of 300-350 V. The medium in the wells was not exchanged post transfection, but an additional 50 μ l of fresh medium was added.

Figure 2



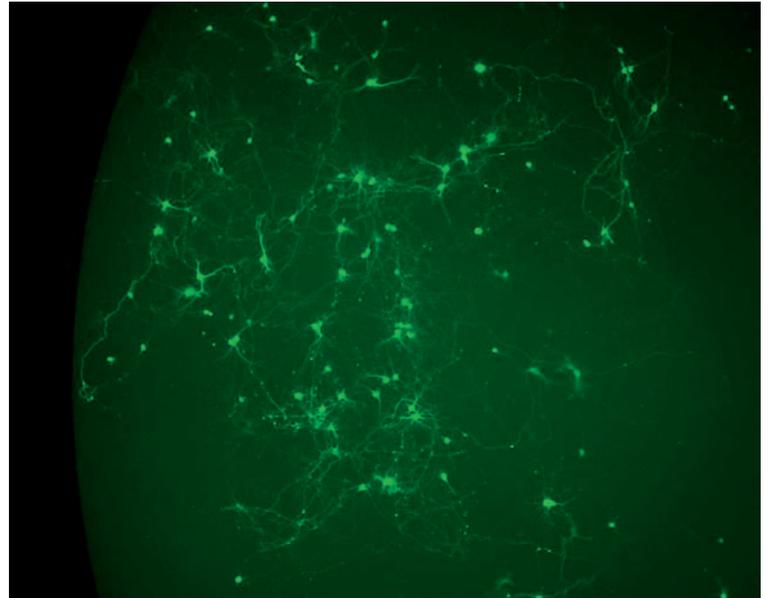
Fluorescence micrograph showing the typical transfection efficiency and specificity of transfection at 48 hours. Neurons are stained for MAP2 (red) and expression of CopGFP is shown in green.

Materials and methods

Cell culture. Primary dissociated hippocampal neurons from rat (E18) were received cryopreserved in vials (Lonza, Cat. no. R-Hi-501) and were stored in liquid nitrogen until used. On the day of culture the cells were thawed in 37°C water bath for 1 minute. Pre-warmed cell culture medium (Neurobasal medium, 1% Penicillin/Streptomycin, 1% L-glutamine, 2% NSF-1, (PNGM) BulletKit®, Lonza) was added to the cells drop-wise while gently rotating the tube in order to avoid osmotic chock. Cells were subsequently plated in a 96-well plate pre-coated with poly-D-lysine (Greiner BioOne cat. no. 655946) to a final density of 27,000 neurons/well, based on the manufacturers statement that the vial contained 1,000,000 cells. The cells were allowed to attach in 37°C and 5% CO₂ for 4 hours, after which all but 35 µl of the plating medium was removed, and fresh medium was added to a final volume of 150 µl. Cultures were maintained at 37°C and 5% CO₂ for at least 7 days, with a 50% medium exchange every 5 days.

Immunocytochemistry. Immunostaining was performed neuron specific for Microtubule-Associated Protein 2 (MAP2), and glial specific for Glial Fibrillary Acidic Protein (GFAP) to assess the ratio of neurons to glial cells in each culture. Cells were fixed at 48 hours post transfection by removing the medium and applying fixing solution (PBS, 4% paraformaldehyde) for 15 minutes at room temperature. Cells were washed twice with PBS before application of permeabilization buffer (PBS, 1% normal goat serum, 0,25% Triton X-100) for 20 minutes at room temperature. Primary antibodies were diluted in permeabilization buffer as; mouse anti-MAP2 IgG (Santa Cruz Biotechnology, sc-32791) dilution 1:50, and rabbit anti-GFAP IgG (Sigma, G9269) dilution 1:400 and added to the wells. The plate was incubated for 1 h at 37°C. The cells were washed with wash solution (PBS, 0,25% Triton X-100) twice. Secondary antibodies were diluted in permeabilization buffer as; FITC-conjugated goat anti-mouse IgG1 (Santa Cruz Biotechnology, sc-2078) 1:100 and Rhodamine Red-X-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch) 1:400 and added to the wells. Plates were incubated in darkness for 1 hour at room temperature. The wells were then washed with wash solution and stored in PBS at 4°C until examined by fluorescence microscopy.

Figure 3



5x fluorescence micrograph of CopGFP expression in 12 day old neurons, showing the typical amount of transfected cells in the well of a 96-well plate at the plating density recommended by the cell supplier.

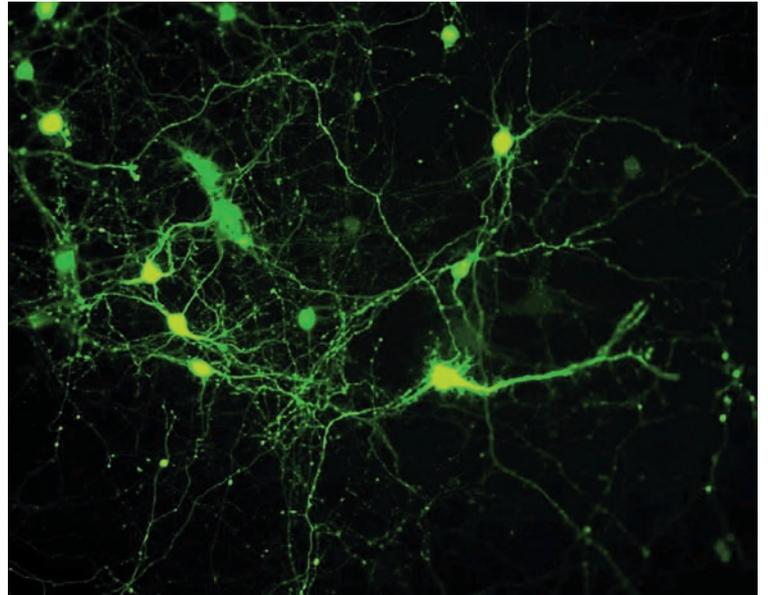
Conclusions

The field of neuroscience presently lacks simple and efficient methods for transfection of primary dissociated hippocampal neurons. We believe that we address a significant bottleneck in neuroscience research, and that this can prove to be a truly enabling method for transfection of developed networks of primary neurons.

References

1. Berry M, Barrett L, Seymour L, Baird A, Logan A. Gene therapy for central nervous system repair. *Curr Opin Mol Ther* 2001;3:338-49.
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3. Wu P, Phillips MI, Bui J, Terwilliger EF. Adeno-associated virus vector-mediated transgene integration into neurons and other nondividing cell targets. *J Virol* 1998;72:5919-26.
4. Nilsson M, von Bonsdorff CH, Weclawicz K, Cohen J, Svensson L. Assembly of viroplasm and virus-like particles of rotavirus by a Semliki Forest virus replicon. *Virology* 1998;242:255-65.

Figure 4



20x fluorescence micrograph of CopGFP expression in 12 day old neurons. Note the high degree of network development, and the healthy morphology of the CopGFP expressing cells.

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