

Sympathetic neurons in culture

Cell type:
Rat sympathetic neurons

System:
Cellaxess ACE

Data courtesy of MRC LMCB and Dept. of Neuroscience, Physiology and Pharmacology, University College London

Highly efficient transfection of differentiated sympathetic neurons

Stable transfection for up to four weeks after transfection

High cell viability

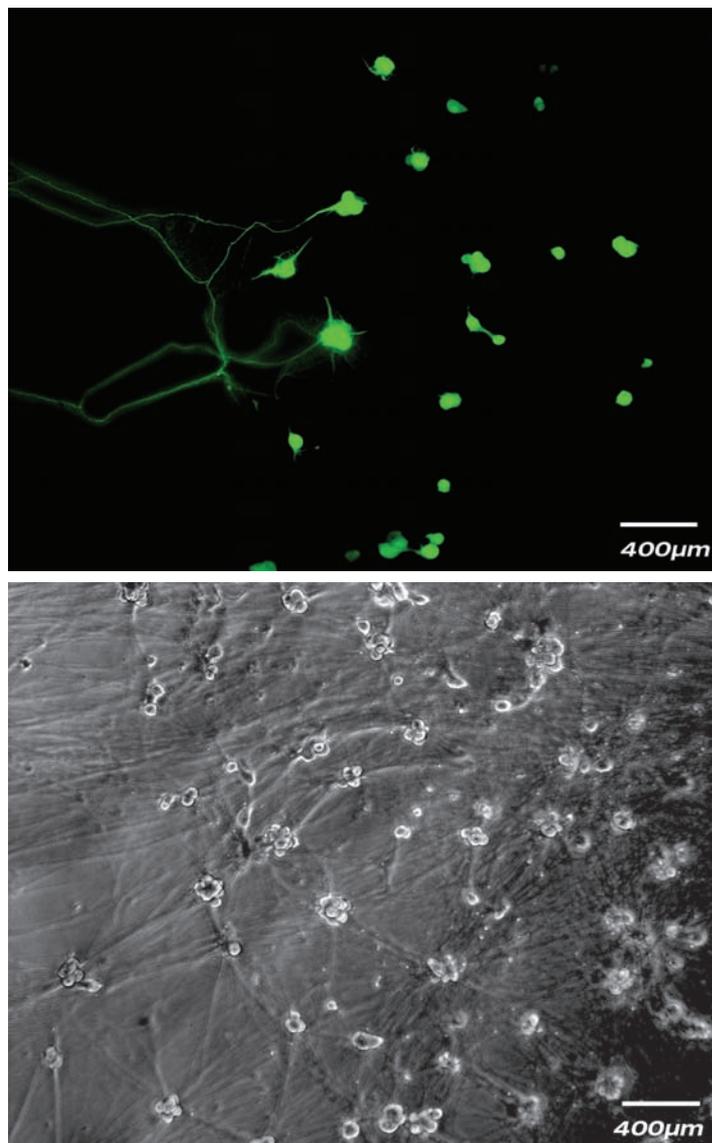
Introduction

Rat sympathetic neurons can be cultured as a highly homogeneous population and are a well-established model to study several aspects of neuronal development. While these primary cultures are extensively used in cell biology and biochemical studies, their use in gene expression studies is hampered by the difficulty of transfecting them. Sympathetic neurons can be transfected by microinjection technique and adenovirus infection. However, these techniques require skilled operators and are often lengthy and cumbersome. On the other hand, lipid-based transfection reagents have repeatedly proved to be toxic or not effective. Here, we report the use of Cellaxess ACE to transfect primary sympathetic neurons with high efficiency without any adverse effect on cell viability.

Results

GFP expression was detectable 4 hours after transfection (Fig. 1). Efficiency of transfection was 40% measured by visual observation under fluorescent microscope. The cell viability was 80% as assessed by contrast phase microscopy. Sympathetic neurons express very intense GFP fluorescence in cell bodies and axons for up to four weeks in culture without apparent loss of viability (Fig. 2).

Figure 1



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Transfection with Cellaxess ACE system

Primary cultures of rat SCGs were maintained in vitro for 4-6 days, and transfected with endotoxin-free plasmid DNA encoding green fluorescent protein (pEGFP-C1, Clontech). The DNA was diluted at 200 ng/μl concentration in basal medium (DMEM alone). Cells were removed from the incubator and the culture medium was exchanged to DMEM containing 100 ng/ml NGF only. The transfection solution 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 was returned to the incubator.

Cell culture

Superior Cervical Ganglia (SCGs) were dissected from 1-2 days old Wistar rat and cultured as previously described (Mains and Patterson, 1973; Riccio et al., 1997). Briefly, ganglia were freed from surrounding tissue and digested in Solution I (4 mg/ml Hyaluronidase, 1 mg/ml collagenase, 0.6 mg/ml DNase, 10 mg/ml fat-free BSA in Hank's Balanced Saline Solution containing 12 mM glucose and 15 mM HEPES, pH 7.4) for 20 min at 37°C. After centrifugation for 5 min at 800 rpm at room temperature, the supernatant was removed and the tissue digested in Solution II (3 mg/ml trypsin in Hank's Balanced Saline Solution with glucose and HEPES as above) for 15 min at 37°C.

Undigested tissue was triturated with a glass Pasteur pipette and passed through a cell strainer (Falcon) to obtain a single cell suspension. Cells were centrifuged for 10 min at 1000 rpm, at room temperature and resuspended in cell culture medium (DMEM 4.5 g/l glucose, 10% FBS, 4 mM glutamine, antibiotics, 100 ng/ml NGF). 15000 cells/dish were plated on 12 mm collagen-laminin coated coverslips (each positioned in a well of a 4-well plate Nunc) in 80 μl total volume. Cytosine arabinoside (ARA-C, 10 μM) was added 24 hours after plating to block proliferation of non-neuronal cells. Culture medium was changed every 2-3 days with addition of fresh NGF

References

- R.E. Mains and P.H. Patterson, *J. Cell Biol.*, 59, 329 (1973)
A. Riccio, B.A. Pierchala, C.L. Ciarallo and D.D. Ginty, *Science*, 277, 1097 (1997)

Figure 2

