Cellaxess® ACE

Explants of sympathetic neurons

Cell type: Explants of rat sympathetic neurons

System: Cellaxess ACE

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

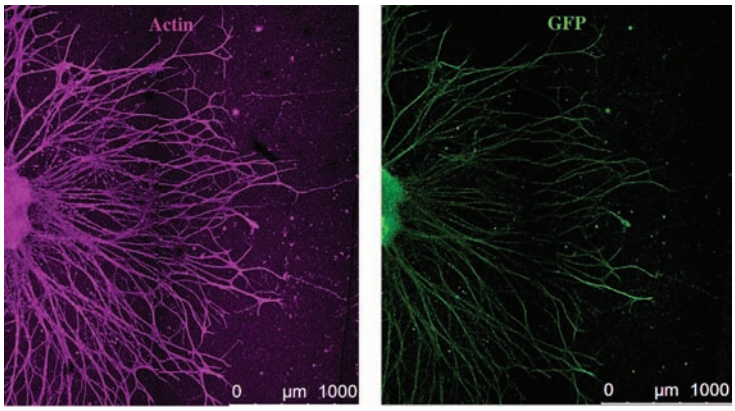
High efficiency transfection of SCG explants for studying axonal outgrowth

Co-transfection of plasmid DNA and siRNA in sympathetic neurons

Introduction

Explant cultures of superior sympathetic ganglia (SCG) have been extensively used to study axonal elongation and targeting as they can be easily dissected from various animal models including newborn and adult rodents, and avian and amphibian sources. SCG explants grow very rapidly forming a dense halo of axons within 18-24 hrs after plating. SCG explants have also been used to investigate the molecular interactions between out-growing axons and myelin-producing Swann cells. The use of this culture model however, would greatly benefit from efficient transfection methods. Similarly to dissociated cultures of sympathetic neurons, so far only microinjection techniques and adenovirus infection have been provensuccessful in transfecting SCG explants. Here we describe the use of Cellaxess CX1 to transfect plasmid DNA and siRNA in SCG explants obtained from P1 rats, and cultured on glass coverslips.

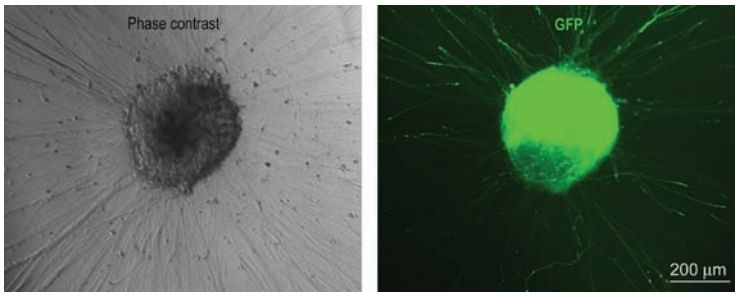
Figure 1



Results

Rat SCG explants were cultured for 1-3 days in vitro and electroporated with the Cellaxess CX1 electroporation system. The expression of green and red reporter fluorescent proteins was clearly detectable 24hrs after electroporation, however maximal expression was observed after 48hrs (Fig.1, 2 and 3). When non-targeting fluorescent labeled siRNA were transfected, fluorescence was visible in cell bodies 3 hrs after transfection (Fig. 4). The percent of successful electroporated explants was of about 75%. Quantification of number of electroporated cells was obtained by counting the number of GFP-positive axons, and was >50 cells/explant. Cell viability was not affected by the electroporation and transfected neurons display intact axons, as assessed by immunostaining for actin (Fig. 1) or by observation under contrast phase microscopy (Fig. 2 and 4)

Figure 2

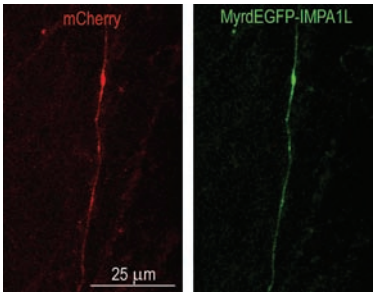


Cellectricon SIMPLY STIMULATING

Transfection with Cellaxess ACE system

Explants from rat SCGs were maintained in vitro for 1 to 3 days and transfected with 200 ng/µl endotoxin-free plasmid DNA encoding green fluorescent fluorescent protein (pEGFP-C1, Clontech), or co-transfected with DNAs encoding the MyrdEGFP-Impa1-L construct (Andreassi et al., 2010) and a red fluorescent protein (mCherry, Clonetch) diluted in basal medium (DMEM alone) at 200 ng/mL and 20 ng/mL, respectively. In separate experiments, explants were electroporated with BLOCK-iT™ Fluorescent Oligo (Invitrogen) diluted to 1mM siRNA in basal medium. Explants were removed from the incubator, culture medium was changed to DMEM containing 100ng/mL NGF and the dishes were placed in the CX1 safety box. The electrode was positioned in a suitable area of the dish, lowered to just barely touch the surface of the explant and electroporation was performed. The electrode head was lifted very carefully, the plate was rotated and a second well was electroporated. The duration of each electroporation was 2.5 min. Four hrs after transfection, medium was changed with complete cell culture medium (see below).

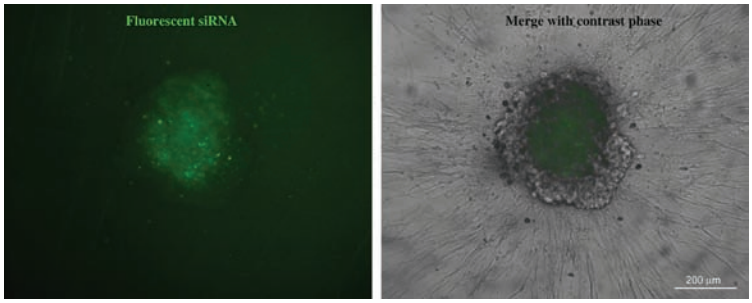
Figure3



Cell culture

Superior Cervical Ganglia (SCGs) were dissected from P1 Wistar rat and cultured, as described (Andreassi et al., 2010). Ganglia were freed from the surrounding tissues and cut in 3 pieces each using a 0.15mm scalpel. Each piece of explant was plated on a 12mm collagen-laminin coated coverslip, positioned in a well of a 4-well plate (Nunc), using 300µl total volume of cell culture medium (DMEM 4.5 g/L glucose, 10% FBS, 4 mM glutamine, antibiotics, 100 ng/mL NGF, 10 mM cytosine arabinoside). Culture medium was changed every 2-3 days with addition of fresh NGF. It should be noted that SCG explants can be cultured also on polyornithine-laminin cotated coverslips, however adhesiveness to this substrate is insufficient to prevent detachment of the explants after electroporation.

Figure 4



References

C. Andreassi, C. Zimmermann, R. Mitter, S. Fusco, S. De Vita, A. Saiardi and A. Riccio, Nature Neuroscience 13, 291–301 (2010).

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Rev. 1.06/2012