Cellaxess® ACE

Transfection of human iPSC-derived neurons

Cell types:
Human iPSC-dervied neurons, directly differentiated & differentiated from expandable precursors

System:
Cellaxess ACE

Data courtesy of the Max Planck Institute for Molecular Biomedicine, Münster, Germany

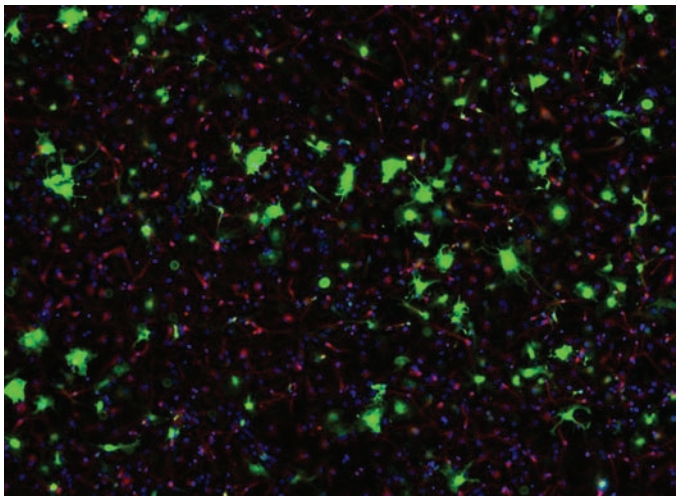
Introduction

Neurons in culture are considered to be a highly relevant in-vitro model in the study of neuronal development and activity. In particular, human iPSC-derived neurons are of special interest when it comes to certain areas, in example the study of Parkinson's disease (PD), where studies have been hindered by the lack of access to human dopaminergic neurons (DA). However, transfection of human iPSC-derived neurons is highly challenging, hampering the research using this highly relevant cell model. With regards to transfection of 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. 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. Here we present Cellaxess ACE for transfection of human iPSC-dervied neurons derived directly or from expandable neural precursors at various stages of differentiation. This enables simple, highly efficient and reproducible transfection without significant impact on the cell viability and morphology.

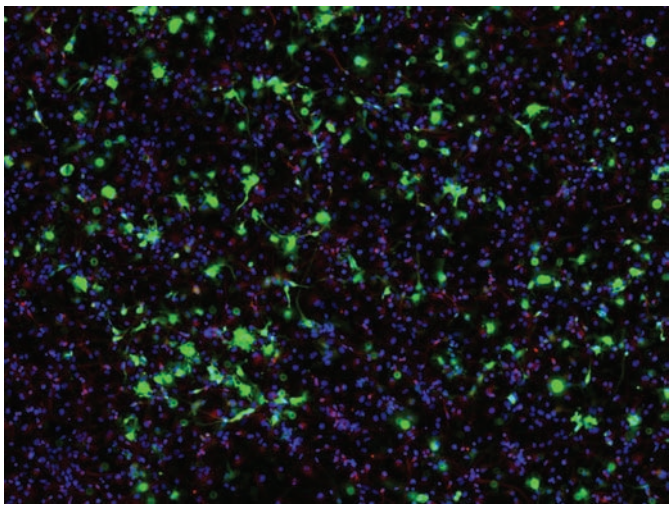
Figure 1

Examples of transfections carried out on cultures plated on Matrigel 96 hours post plating. (a) and (b) shows transfection of directly differentiated cells plated at 25,000 and 50,000 cells/well respectively. (c) shows transfection of a culture differentiated from expandable precursors plated at 50,000 cells/well. This culture appears to contain a higher percentage of mature neurons.

a)



b)



Results

Expression of CopGFP fluorescent protein was evaluated at 24h post transfection by immunocytochemistry and fluorescence microscopy. For cell identification, cultures were fixed and stained with neuron specific (tubulin) and dopaminergic specific (TH) antibodies (not shown). The transfection was neuron specific (although not completely) with up to 40% transfection efficiency for neurons and in general low transfection efficiency for glial cells and precursors using an optimized protocol for neurons. Figure 1 shows examples of typical cultures transfected at 96 hours after plating at 24 hours post transfection. Figure 2 shows examples of typical cultures transfected at 24 hours after plating at 24 hours post transfection. Cells differentiated from the neural expandable precursors appeared to generate somewhat higher percentage of mature neurons compared to those that had been directly differentiated. Furthermore, cells plated in Matrigel-coated wells appeared to spread and attach more evenly in the wells compared to those plated on Cellstart. However, the overall transfection efficiency did not vary greatly depending how they were differentiated or depending on the coating.

When looking at transfection efficiency versus time of transfection after plating, cultures transfected 96 hours post plating in general exhibited somewhat higher transfection efficiencies than those transfected 24 hours post plating, except in the case of 50,000 cells/well plated on Cellstart, where very high neuronal transfection efficiency was achieved on cultures transfected 24 hours post plating (Figure 2d). Estimated maximum transfection efficiency for cultures transfected 96 hours and 24 hours post plating was approximately 40% and 25%, respectively. The viability was high compared to mock transfections, and the morphology indicated that cells remained healthy after transfection. Little or no difference could be seen morphologically between mock and transfected wells.

Transfection using 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, Moscow, Russia) at 100 ng/µl 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 420 V. The medium in the wells was exchanged 2-4 hours post transfection.

1 c)

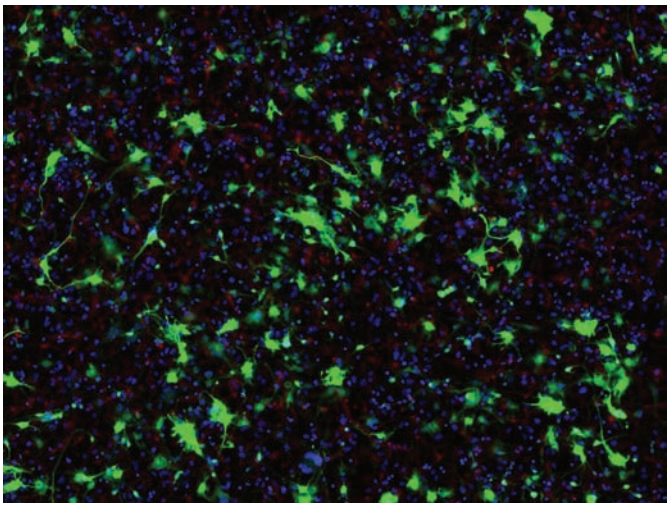
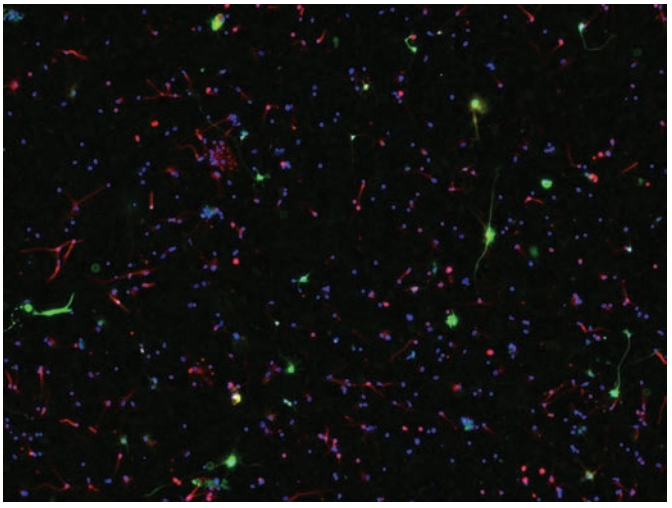


Figure 2

Examples of transfections carried out on cultures 24 hours post plating. (a) and (b) show examples of cells plated on Matrigel at a density of 25,000 cells/well, (a) showing directly differentiated and (b) showing cells differentiated from neural precursors. There appears to be a higher percentage of non-neuronal cells in the culture that has been directly differentiated. (c) and (d) shows examples of cultures differentiated from expandable precursors, (c) being plated at a density of 25,000 cells/well and (d) at 50,000 cells/well. Note the high transfection efficiency of mature neurons in these cultures.

a)



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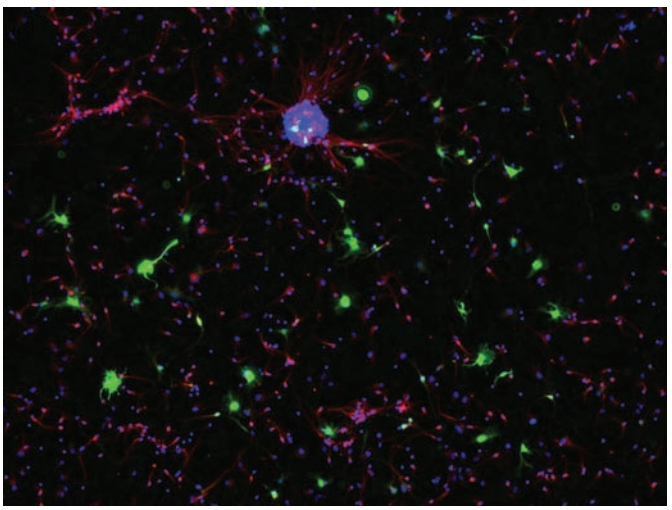


Materials and Methods

Cell culture:

Human iPSC-derived cultures containing a mix of dopaminergic neurons, non-dopaminergic neurons, glial cells and precursors were generated directly from human skin fibroblasts according to (1) or from expandable neural precursors (unpublished data). 24 or 96 hours prior to transfection, cells were dissociated from the flasks in which they were cultured by a combination of enzymatic and gentle mechanical methods, and plated into 96-well plates at different densities (25,000 & 50,000 cells/well). Different coatings were also evaluated for both time points and densities (Matrigel and Cellstart).

Immunocytochemistry:

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 with 0.1% Triton X-100, 10% FCS and 1% BSA) for 45 minutes at room temperature. Antibodies subsequently applied were mouse anti TUJ1 (beta-3-Tubulin) from Covance 1:2000, rabbit anti TH from PelFreez 1:500, in PBNS with 0.1% BSA overnight. Cell were then washed with PBS stained with secondary antibodies (Alexa, Life Technologies, Carlsbad, CA, USA) for one hour at room temperature in PSA/BSA and finally washed and counterstained with Hoechst. The plates were then washed with wash solution and stored in PBS at 4°C until examined by fluorescence microscopy.

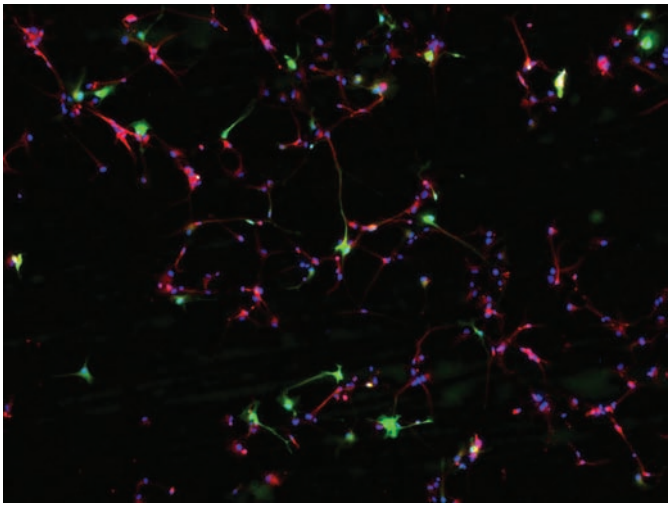
Conclusions

The field of neuroscience presently lacks simple and efficient methods for transfection of primary dissociated neurons, in particular when it comes to human iPSC-derived neurons in various stages of differentiation. 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 human iPSC-derived neurons.

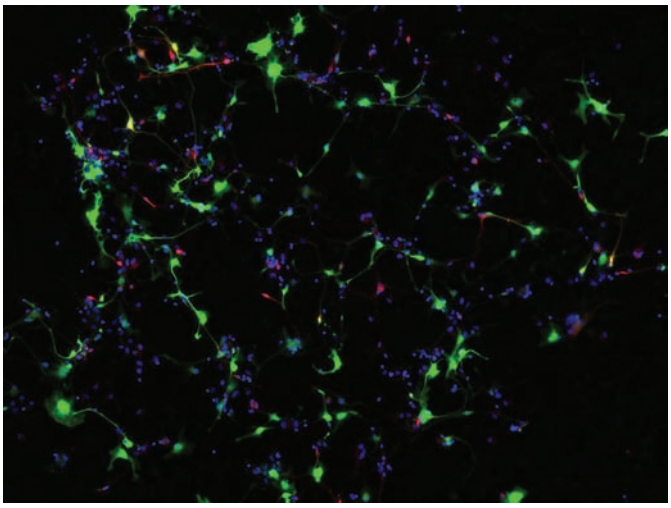
References

1. Cell Stem Cell 8, 267–280, March 4, 2011

2c)



2d)



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