Benchmarks

High transfection efficiency of neural stem cells with magnetofection

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Primary neural stem cells (NSCs) can be cultivated and differentiated in vitro but are difficult to transfect using conventional methods. We describe a simple and rapid magnetofection-based method suitable for the lab bench as well as for high-throughput projects. Our method yields high transfection efficiency and can be used for deciphering the genetic control of neural cell differentiation.

The incidence of neurodegenerative diseases has increased exponentially, yet very few therapeutic options are available for treating these pathologies. One option consists of replacing lost cells, either by mobilization of endogenous neural stem cells, or by grafting exogenous stem cells previously induced to differentiate toward the intended cell type. A common requirement for applying these strategies is an understanding of the processes leading from neural stem cells (NSCs) to differentiated neural cells.

NSCs can be isolated from postnatal mouse brains and cultivated as neurospheres in vitro. These are clonal aggregates of neural stem cells that can be induced to differentiate by growth factor removal. Due to their multipotent properties, NSCs can differentiate into each of the three neural cell types: neurons, astrocytes, and oligodendrocytes. However, the genetic control of these differentiation processes is still poorly understood and the differentiation toward a specific cell type cannot be easily controlled. Moreover, it is currently impossible to induce their differentiation into a specific neuronal subtype, which would be necessary to apply these techniques in therapy (e.g., the differentiation into dopaminergic neurons in the context of Parkinson's disease). One potent strategy to decipher the genetic control of differentiation is to perform large-scale genetic studies based on the systematic transfection of listed genes in order to identify candidates that either induce or suppress differentiation into a specific cell type.

The magnetofection technology (1) is very effective in transfecting plasmid DNA into various primary cells including primary neurons. This method is based on the use of a magnetic field that acts

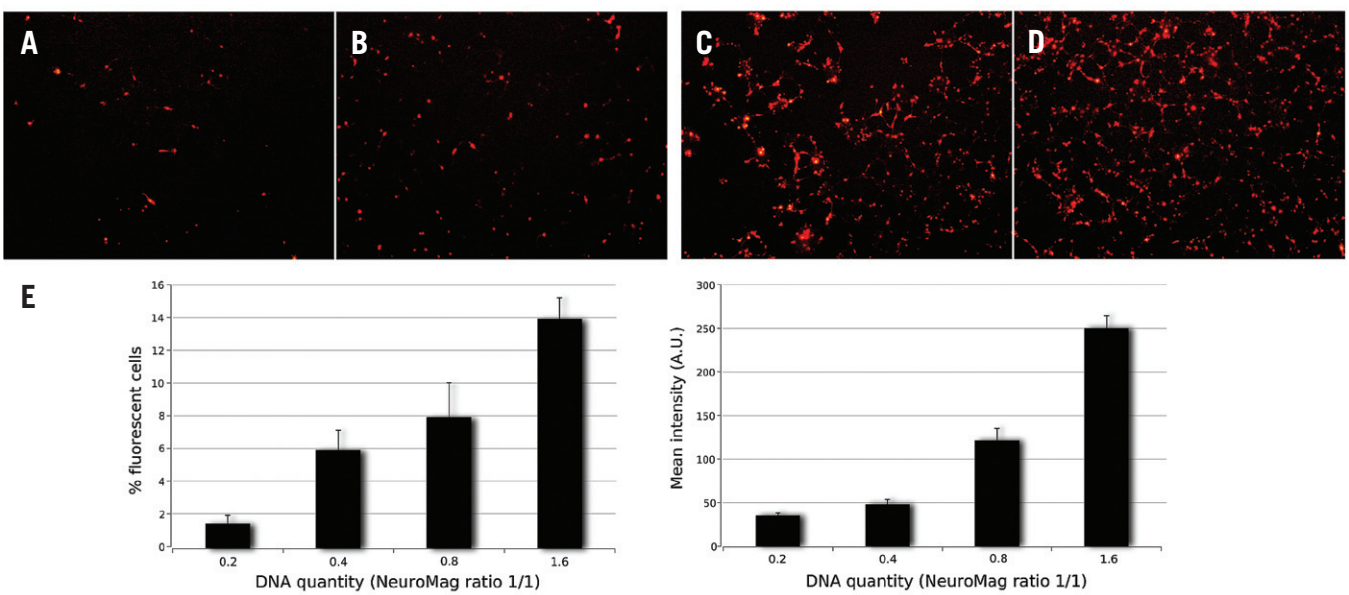

Figure 1. Optimization of NeuroMag transfection efficiency in mouse NSCs. (A–D) Fluorescent images of Neural Stem Cells cultured in a 24-wells plate transfected with 0.2 µg (A), 0.4 µg (B), 0.8 µg (C), and 1.6 µg (D) DsRed plasmid mixed with NeuroMag reagent in a 1:1 ratio. (E) Percentage of GFP-positive cells (upper graph) and mean fluorescence intensity (lower graph) 2 days after transfection with different amounts of DNA/NeuroMag complexes at a 1:1 ratio. For transfection, NeuroMag reagent was mixed with DNA in a volume of culture medium of 50 µL per transfected well. Complexes of magnetic nanoparticles and DNA were allowed to form via 15 min of incubation at room temperature before addition into the wells. The culture plate was then placed on a magnet for 15 min. Cells were incubated in the presence of NeuroMag for 1 day before changing the medium.

on nucleic acid vectors associated with superparamagnetic nanoparticles. The magnetic force leads to a rapid accumulation of the vector around cells/organs and promotes cellular uptake. While minimizing undesired toxic effects associated with other transfection methods, a specific magnetic nanoparticle formula called NeuroMag (OZ Biosciences, Marseille, France), with beads ranging in size from 140 to 200 nm and a positive zeta potential, has proven to be extremely efficient in transfecting a large variety of primary neurons such as cerebellar granule cells (2,3), dorsal root ganglia (4), hippocampal neurons (5–7), and motor neurons (8). Moreover, high transfection efficiency was also achieved in primary rat astrocytes from cerebral cortices (9,10).

Here we show that the NeuroMag reagent enables efficient gene delivery into NSCs without affecting their differentiation.

Neurosphere culture was initiated from P1 mice subventricular zone (SVZ) and was performed as previously described (11,12). Cells were cultured in the presence of 20 µg/mL EGF and bFGF to maintain an immature state. For transfection, cells at passage 2 were dissociated and seeded in adherent conditions on polyornithine-coated wells at a density of 250,000 cells per well of a 24-well plate. One day later, transfection was performed using NeuroMag reagent and a Super Magnetic Plate (OZ Biosciences). To optimize the transfection procedure, we used a DsRed-expressing plasmid (Clontech, Mountain View, CA, USA) and transfection efficiency was measured 2 days post-transfection by counting the percentage of red cells among total living cells in randomly selected fields (Figure 1). While investigating the transfection efficiency as a function of DNA and NeuroMag amounts, we found that when NeuroMag content (in microliters) was significantly higher than DNA content (in micrograms)—as it is the case in the protocol used for most of the neural cells—high cell toxicity was observed. Consequently, in subsequent experiments the ratio of NeuroMag to DNA was maintained at 1:1. Under these conditions, the transfection efficiency increased with NeuroMag concentration (Figure 1). However, at concentrations >1.6 µL/well, we observed cell death in transfected cells characterized by the progressive detachment of cells from the substrate, which impaired accurate transfection efficiency evaluation. The optimal conditions for culturing cells in 24-well plates were thus found to be 1.6 µL NeuroMag per 1.6 µg DNA, which yielded a transfection efficiency of 15% (Figure 1). A detailed protocol for NeuroMag optimization is available online as Supplementary Material. We also evaluated the use of this transfection method to perform functional analysis aiming at identifying genes involved in the neuronal differentiation of neural stem cells. We have previously shown that neurosphere cells electroporated with a combination of GFP- and NeuroD1-expressing plasmids led to fluorescent cells with an obvious neuronal morphology. These cells were subsequently shown to express the neuronal markers Tuj1 and NeuN (13). However, when the GFP plasmid was electroporated together with an empty vector, the fluorescent cells had an astrocytic phenotype and expressed the glial marker GFAP (13). We therefore transfected neurosphere cells with a mixture of pCX-eGFP– and NeuroD1-expressing plasmids (1:2 mixture, respectively) using the protocol described above, except that transfected cells were cultured in an EGF- and FGF-free medium to induce differentiation. Four days after transfection, we observed that almost all GFP-expressing cells showed a neuronal morphology with long neurites, whereas most of the GFP-expressing cells transfected with the empty vector showed a glial morphology (Figure 2).

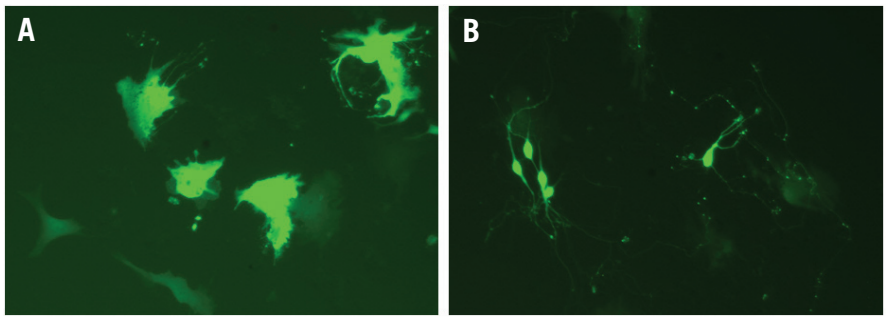

Figure 2. Neural stem cell differentiation. Images of neural stem cells acquired 4 days after NeuroMag transfection of a mix of a GFP vector together with either an empty vector (A) or a NeuroD1-expressing vector (B).

To summarize, we demonstrate that NeuroMag-mediated transfection represents an efficient and very simple method for NSC transfection. This method presents important advantages compared with alternative approaches. Nucleofection (Amaxa, Lonza, Basel, Switzerland), although yielding a higher percentage of healthy transfected cells (30–40% in our conditions), is significantly more costly and most cells die during electroporation. Moreover, all lipofection methods that we tested and which induced high levels of reporter gene expression exhibited extensive toxicity soon after transfection. We have also shown that NeuroMag transfection can be used to analyze the involvement of genes during neuronal differentiation of NSCs. Due to the simplicity and low cost of the method, NeuroMag-mediated transfection is the method of choice to carry out high-throughput projects

for the discovery of new regulators of neuronal differentiation.

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Competing interests

C.S., N.L., L.G., E.B., and O.Z. are employed by OZ Biosciences, which manufactures and distributes the NeuroMag product.

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