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The transfection of multipotent neural precursor/stem cell transplant populations with magnetic nanoparticles

Mark R. Pickard [a], Perrine Barraud [b], Divya M. Chari [a,*]

[a] Cellular and Neural Engineering Group, Institute for Science and Technology in Medicine, Keele University, Keele, Staffordshire ST5 5BG, United Kingdom
[b] Department of Physiology, Development and Neuroscience, University of Cambridge, Anatomy Building, Downing Street, Cambridge CB2 3DY, United Kingdom

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ABSTRACT

Multipotent neural precursor/stem cells (NPCs) are a major transplant population with key properties to promote repair in several neuropathological conditions. Magnetic nanoparticle (MNP)-based vector systems, in turn, offer a combination of key benefits for cell therapies including (i) safety (ii) delivery of therapeutic biomolecules (DNA/siRNA) enhanceable by 'magnetofection' approaches (iii) magnetic cell targeting of MNP-labelled cells to injury sites and (iv) non-invasive imaging of MNP-labelled transplant populations for cell tracking. However, the applications of the versatile MNP platform for NPC transplantation therapies have received limited attention so far. We have evaluated the potential of MNP vectors for gene transfer to NPCs using a neurosphere culture model system; we also assessed repeat transfection ("multifection") and repeat transfection plus applied magnetic field ("magneto-multifection") strategies [to enhance transfection efficiency]. We show for the first time that MNPs can safely mediate single/combinatorial gene delivery to NPCs. Multifection approaches significantly enhanced transfection with negligible toxicity; no adverse effects were observed on stem cell proliferation/differentiation. "Multifected" NPCs survived and differentiated in 3D neural tissue arrays post-transplantation. Our findings demonstrate that MNPs offer a simple and robust alternative to the viral vector systems currently used widely to transfect neural stem cells in neurobiology/neural transplantation research.

1. Introduction

The adult central nervous system (CNS), comprising the brain and spinal cord, is capable of limited regeneration so clinical recovery is frequently poor in neurological injury/disease. Research in experimental neurology has identified several neurotherapeutic molecules (e.g. a range of growth factors) that promote CNS repair; expression of key repair-promoting genes in trauma sites therefore constitutes a major therapeutic strategy to promote neuroregeneration [1–7], but a major challenge confronting CNS gene therapy is the 'translational' one [8,9] of developing methodologies that will allow for safe and effective delivery of therapeutic biomolecules to such sites.

Multipotent neural precursor/stem cells (NPCs) have major applications in this context; the role of NPCs as a cell source for 'restorative cell therapeutics' in areas of neural trauma is well documented (given their dual properties of continuous self-renewal and multipotentiality [ability to differentiate into the three major CNS cell types – neurons, astrocytes and oligodendrocytes]) [10–13]. Additionally, NPCs possess key properties that make them attractive candidates to function as 'vehicular biopumps' for delivery of therapeutic molecules to areas of CNS injury [14] – NPCs can migrate long distances in host CNS tissue and, significantly, tend to migrate towards areas of CNS pathology (e.g. tumours or neurodegenerative foci); a phenomenon termed pathotropism. NPCs introduced intravenously migrate across the blood–brain barrier (that normally poses a critical barrier to CNS biomolecule delivery) in response to ongoing inflammation, reactive astrocytosis and angiogenesis to enter injury sites [15], and integrate into host tissue with little functional disruption. Consequently, NPC-mediated gene delivery is suggested to be more efficient (compared with other neural transplant populations) with minimal side effects exerted in surrounding normal neural tissue [14]. NPCs are expandable for long periods in vitro (making them amenable to genetic engineering techniques) and the beneficial neurological effects of NPC-mediated gene transfer in sites of neural pathology are established, so it is widely considered that NPCs can aid in safe delivery of therapeutic molecules to diseased/injured neural tissue.

It should be noted here that most studies to date have employed virus-mediated gene delivery to transduce NPCs [16,17] – although

* Corresponding author. Fax: +44 1782 734634.
E-mail address: d.chari@hfac.keele.co.uk (D.M. Chari).

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highly efficient, viral vectors have several drawbacks, including safety issues such as neurotoxicity, non-specific cellular uptake and induction of immune and inflammatory responses [8,14]. Insertional mutagenesis and oncogenic effects have been observed in vivo, leading to abnormal cellular growth, with frequent transgene silencing. Viral gene transfer also involves significant technical complexity and there are major methodological limitations with viral delivery in terms of limited plasmid insert size and achieving large-scale production.

The above drawbacks have resulted in the major current international drive for development and optimisation of alternative non-viral vectors for biomedical applications [18]. In this context, recent research has demonstrated a unique combination of advantages offered by magnetic nanoparticle (MNP)-based vector systems for cell transplantation [19–21]. MNPs have a magnetic core with biocompatible surface coating that can be 'functionalised' for delivery of therapeutic biomolecules (DNA, siRNA and shRNA) – MNP size and surface chemistry are adaptable to suit the functionalising molecule which is increasingly allowing for development of tailored particles for specific biomedical applications. For cell replacement studies, it is vital to track transplant populations long-term to evaluate outcomes of cell transplantation procedures and correlate these with functional neurological recovery – MNPs are routinely used as MRI contrast agents for CNS imaging, and clinical trials support the notion that MNP delivery systems are adaptable for therapeutic use [22]. MNPs do not alter the viability/differentiation potential of neural transplant populations including Schwann cells, olfactory ensheathing cells and oligodendrocyte progenitor cells (OPCs) [23,24]; one study shows that MNP labelling of human neural precursor/stem cells (grown as neurospheres) does not alter cell survival, migration, differentiation or electrophysiological characteristics [25], suggesting that MNPs persist within transplanted NPCs with limited long-term cytotoxicity or adverse effects on cell function. Notably, the magnetic properties of MNPs allow for targeted delivery of MNP-labelled cells (introduced systemically) by applying high gradient magnetic fields over target tissues [26], potentially providing a minimally invasive magnetic cell targeting strategy to concentrate transplanted cells at sites such as the spinal cord [26].

We recently tested magnetic particles for a range of applications related to transplantation of astrocytes (a major neural cell transplant population) and demonstrated the significant potential of MNPs for cell tracking and transfection of astrocytes derived for transplantation [27,28]; transfection efficacies were dramatically improved using 'magnetofection' approaches (use of static/oscillating magnetic fields to enhance transfection) [27]. Based on our findings, we suggested that MNPs show significant promise to serve as a "multifunctional nanoplatform" for neural cell transplantation therapies [27,28]. To date, however, the use of MNPs to mediate safe gene transfer to the major transplant population of multipotent NPCs has not been assessed. We address this issue in the current study using cultures of multipotent NPCs (neurosphere model). Our primary objectives were to: (i) prove the feasibility of MNP-mediated transfection of NPCs (single and combinatorial gene transfer); (ii) assess if transfection can be enhanced by "multifection" and "magneto-multifection" strategies; (iii) assess survival and differentiation potential of NPCs transfected with MNPs to establish the safety of transfection protocols used; and (iv) assess transplantation potential of transfected NPCs in host, organotypic cerebellar slice cultures.

2. Materials and methods

2.1. Reagents

Cell culture reagents were from Invitrogen (Paisley, Scotland, UK) and Sigma (Poole, Dorset, UK). Human recombinant basic fibroblast growth factor (bFGF) was also from Sigma (Poole, Dorset, UK) and human recombinant epidermal growth factor (EGF) from R&D Systems Europe Ltd. (Abingdon, UK). Thermo Scientific Nunc culture dishes (non-treated surface) and tissue culture-grade plastics were from Fisher Scientific UK (Loughborough, UK). Millicell culture inserts and Omnipore membrane were from Millipore (Watford, UK). Neuromag MNPs were purchased from Oz Biosciences (Marseille, France); Neuromag particles are positively charged with average particle size 160 nm (range 140–200 nm) and appear homogenous in shape/size under scanning electron microscopy [27]. We previously assessed particle size before and after pmaxGFP plasmid conjugation using a Malvern Zetasizer 3000; binding of plasmid to the particles results in an approximate 35% increase in particle size [27]. pmaxGFP plasmid was from Amaxa Biosciences (Cologne, Germany) and pCMV DsRed-Express2 plasmid was from Clontech (Saint-Germain-en-Laye, France). The magnefect-nano 24-magnet array system was purchased from nanoTherics Ltd. (Stoke-on-Trent, UK) and comprises horizontal arrays of NdFeB magnets (grade N42) onto which 24-well cell culture plates can be placed. The following antibodies were used: anti-nestin (clone 25, BD Biosciences, Oxford, UK), rabbit anti-SOX-2 (Millipore, Watford, UK), neuronal class III β-tubulin antibody (clone TUJ1, Covance, Princeton, NJ), myelin basic protein (MBP) (clone 12, AbD Serotec, Kidlington, UK) and rabbit anti-glial fibrillary acidic protein (GFAP) (DakoCytomation, Ely, UK). Cy3- and FITC-conjugated AffiniPure secondary antibodies were from Jackson ImmunoResearch Laboratoratories Inc. (West Grove, PA, USA), and Vectashield mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) from Vector Laboratories (Peterborough, UK). The care and use of all animals used in the production of cell cultures were in accordance with the Animals Scientific Procedures Act of 1986 (UK).

2.2. Neurosphere culture

NPCs were expanded under growth factor (GF) stimulation according to the well characterised 'neurosphere' culture method – with appropriate plating densities and GF stimulation, continued proliferation by NPCs generates spherical clusters of cells that are commonly termed 'neurospheres' which provide an excellent experimental system to study the proliferation/differentiation of CNS precursor cells and represents the method of choice for their quick and reliable isolation. Neurospheres from the subventricular zone of neonatal CD1 mice were prepared and maintained in medium comprising a 1:3 mix of DMEM:F12 containing 2% B-27 supplement, 50 U/ml penicillin, 50 μg/ml streptomycin, 4 ng/ml heparin, 20 ng/ml bFGF and 20 ng/ml EGF, as described earlier [29]. Cultures were fed every 2–3 days and neurospheres were passaged weekly following dissociation with a mix of accutase-DNase I.

2.3. MNP-mediated transfection of neurospheres

2.3.1. Single transfection

For transfection with MNPs, dissociated cells (passages 2–3) were plated (0.5 ml of 10^5 cells/ml) in 24-well plates and cultured for 24 h. In initial experiments to assess the MNP-related dose-dependency of transfection, complexes were formed in DMEM:F12 (3:1) base medium and contained (per ml) various volumes of Neuromag (4.4–26.3 μl) and pmaxGFP plasmid at a constant MNP:DNA ratio of 3.5 μl/μg (i.e. the maximal DNA binding capacity of Neuromag as established previously) [27]. Complexes were allowed to form for 20 min at room temperature (RT) then 0.1 ml was added drop-wise to neurospheres whilst gently mixing. Spheres were cultured for 48 h ± a static magnetic field (24-magnet array; Nanotherics) for the first 30 min. Controls were treated with pmaxGFP (highest concentration) alone to establish whether unconjugated plasmids alone could mediate transfection.

2.3.2. Multifection and magneto-multifection

To assess if MNP-mediated transfection could be enhanced by repeat administration of MNP-gene complexes, 'multifection' experiments were conducted. In these experiments, complexes comprised 8.7 μl Neuromag and 2.5 μg DNA per ml DMEM:F12 (3:1) base (prepared as above), and 50 μl complexes were added drop-wise to spheres. For multifection, freshly prepared complexes were added at 24 h (day 1; d1) and 48 h (day2; d2) post-plating (with no exposure to magnetic fields). Control groups comprised: spheres transfected at 24 h only; spheres transfected at 48 h only; and, to assess cytotoxicity associated with these procedures, spheres treated with plasmid alone at 24 h only, 48 h only and 24 h plus 48 h. All neurospheres were harvested at 96 h (i.e. 48 h after the second round of transfection).

The effect of multifection in the presence of a magnetic field (herein termed 'magneto-multifection') was also examined, since we reasoned that field application may increase the sedimentation of MNP-containing spheres from the first round of transfection, thereby increasing cell – particle contact on the bottom of the culture dish (in the second round of transfection) and, consequently, enhance transfection efficiency. These experiments were conducted on sister cultures of those used for multifection and were performed at the same time as the multifection experiments. They employed an identical protocol (and treatment/control groups) to that used for multifection except that a static magnetic field was applied for the first 30 min after each complex (or plasmid only) addition. As for multifection, all neurospheres were harvested at 96 h (i.e. 48 h after the second round of transfection).

2.3.3. Combinatorial gene delivery

Experiments to assess whether MNPs can mediate combinatorial gene delivery employed the plasmid pCMV DsRed-Express2 (encodes a red fluorescent protein