RESEARCH NOTE

Improved transfection of HUVEC and MEF cells using DNA complexes with magnetic nanoparticles in an oscillating field

JENSON LIM[1]* and JON DOBSON[2,3]

[1]*nanoTherics Limited, Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK*
[2]*J. Crayton Pruitt Family Department of Biomedical Engineering and Department of Materials Science and Engineering, University of Florida, Gainesville, FL 32611, USA*
[3]*Institute for Science and Technology in Medicine, Keele University, Stoke-on-Trent, ST4 7QB, UK*

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Introduction

Cells such as mouse embryonic fibroblasts (MEFs) and human umbilical vein endothelial cells (HUVECs) used in stem cell research and endothelial cell physiology and pathology studies are difficult to transfect using 'standard' nonviral transfection methods. We have developed a novel gene delivery technique, which uses magnetic nanoparticles under the influence of an oscillating magnetic array. Here we report results from this technique showing significantly higher transfection efficiency in these cells compared to a static magnetic array and lipid reagents, with no effect on cell viabilities. Further in HUVECs, we report increasing gene transfection and expression with increasing oscillation frequencies and in serum-starved conditions.

Many highly differentiated mammalian cells like neurons or leucocytes, are resistant to the introduction of foreign genes. Various techniques have been developed over the decades to overcome this problem, from use of the relatively cheap lipid-based reagents to the costly nucleofection methods. Magnetofection technology is an effective nonviral transfection tool for the introduction of nucleic acids into mammalian cells. This involves, first association of nucleic acids with polymer-coated superparamagnetic iron oxide nanoparticles. Next, these complexes are subjected to a proprietary novel, strong high-gradient magnet array underlying the cell culture plate that pulls the complexes onto the surface of the cells (Plank et al. 2003; Dobson 2006). In order to improve transfection efficiency in hard-to-transfect cells while maintaining the inherent advantages of magnetofection (rapid transfection and high cell viability), we have introduced a linear oscillating motion to the magnet array to mechanically stimulate the cells, possibly promoting endocytosis, thereby enhancing uptake of the nanoparticle/DNA complex (figure 1).

Our initial studies of this technology demonstrate that increased luciferase enzyme production was successfully achieved in the transfection of a relatively easy-to-transfect cell line, NCI-H292 (human lung epithelial cells), while cell viability was maintained. The technique demonstrated significantly higher luciferase expression with negligible cell death, compared with a static magnet system and two commercially available lipid-based reagents. Transfection efficiencies were also shown to be dependent on magnetic field, frequency and amplitude, with 40 mT, 2 Hz and 0.2 mm shown to be the optimal parameters for NCI-H292 cells (McBain *et al.* 2008; Fouriki *et al.* 2010). This technology subsequently has been demonstrated successfully in several highly differentiated, hard-to-transfect cell types, including purified primary rat astrocytes (Pickard and Chari 2010), primary rat oligodendrocyte precursor cells (Jenkins *et al.* 2011) and primary cardiac progenitor cells (M. Subramanian, J.Lim and J. Dobson unpublished data), with little detrimental effect on cell viability, migration, proliferation and differentiation. It also has potential therapeutic applications (Muthana *et al.* 2008).

Here, we show successful transfection of MEFs and HUVECs using the oscillating magnet array system. Our system significantly outperformed a leading lipid reagent and static magnet array in transfection efficiency, protein overexpression and cell viability. In HUVECs, increasing transfection and expression were linked to increasing frequency of oscillation with the magnet array.

*For correspondence. E-mail: jenson@nanotherics.com.

Keywords. magnetic nanoparticles; transfection; mouse embryonic fibroblasts; human umbilical vein endothelial cells; oscillating magnet array; magnetofection.

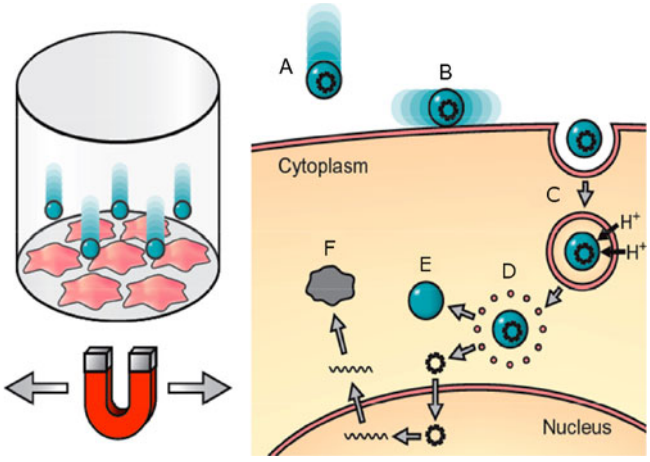

Figure 1. Principle of oscillating nanomagnetic transfection. Plasmid DNA or siRNA is attached to magnetic nanoparticles and incubated with cells in culture (left). An oscillating magnet array below the surface of the cell culture plate pulls the particles into contact with the cell membrane (A) and drags the particles from side to side across the cells (B), mechanically stimulating endocytosis (C). Once the particle/DNA complex is endocytosed, proton sponge effects rupture the endosome (D) releasing the DNA (E) which then transcribes the target protein (F) (after Fouriki *et al.* 2010).

Materials and methods

Cell and reagents

Eukaryotic expression vector pEGFP-N1 (CMV promoter driving gene encoding green fluorescence) was purchased from Clontech (Mountain View, USA). Plasmid DNA was prepared using the Qiagen EndoFree Plasmid Purification kit (Crawley, UK), and maintained in endonuclease-free water (Sigma, Dorset, UK) at −80°C. Mitotically inactivated MEFs isolated from E13.5 CF1 embryos were purchased from R&D Systems (Abingdon, UK) and maintained in the antibiotic-free medium consisting of high glucose DMEM, 10% foetal bovine serum and 2 mM L-glutamine (Biosera, East Sussex, UK). Pooled source of low passage HUVECs with its accompanying recommended Endothelial Cell Growth Medium Package were purchased from TCS Cell Works (Buckingham, UK).

Transfection of MEF and HUVEC

Twenty-four-well plates containing 10,000 MEFs or 60,000 HUVECs per well were incubated at 37°C, 5% CO_2, for 24 h. Some wells containing HUVECs were serum starved for 2 h prior to transfection. Endotoxin-free plasmid pEGFP-N1 1.0 μg or 1.2 μg, was mixed with 1.5 μL or 1.2 μL nTMag (nanoTherics, Stoke-On-Trent, UK). Fe_3O_4 dispersed in a polyethylenimine – HCl matrix; zeta potential: +23.4 mV; particle size distribution: 1.8 (polydisperse index) in 100 μL serum-free medium for MEFs and HUVECs, respectively. These concentrations were found to have little effect on cell viabilities (data not shown). After 20 min, 500 μL serum-containing medium was added to nTMag-GFP complexes and the entire volume of medium containing complexes were added dropwise to wells with the appropriate cells. The lipid reagent used in this study was Lipofectamine 2000 (Invitrogen, Paisley, UK) and complexes were prepared following the manufacturer's recommended protocol. Control wells had untransfected cells. Plates were incubated for 30 min over (identical) magnetic arrays that were either stationary (static) or moving laterally between 1 and 3 Hz at 0.2 mm amplitude of displacement (oscillating) (magnefect-nano system, nanoTherics, Stoke-On-Trent, UK). After transfection, plates were removed from the magnetic arrays and placed back in the incubator.

Flow cytometry and microscopy analyses

Transfected cells were observed at 24 and 48 h and images taken using fluorescence microscopy, using a 10× objective (Olympus, Essex, UK). As there were no substantial differences in results between 24 and 48 h, microscopy images were captured after 48 h to coincide with flow cytometric analysis. For flow cytometric analysis at 48 h, cells were trypsinized, washed in 0.5% bovine serum albumin / phosphate-buffered saline solution and analysed for the relative fluorescence of gated cells, using a FACSort analyser (Becton Dickinson, Oxford, UK). The basal transfection was set at 1%, based on analysis of the untransfected cells (UT). Median fluorescence intensity of gated cells was determined through the FL1 channel.

Results and discussion

We have shown that the addition of a horizontal/lateral motion to the magnet array (figure 1, left) significantly improves the transfection of easy-to-transfect cell lines (www.nanotherics.com) and a range of highly differentiated, hard-to-transfect cell types mentioned earlier. The work here shows that, for MEFs and HUVECs this improvement in efficiency is frequency dependent. MEFs transfected with GFP and nTMag, using this oscillating magnet array, exhibited cell morphologies and densities similar to the untransfected control, under light/fluorescence microscopy, suggesting high viabilities (figure 2, C&D and data not shown). Flow-cytometric analysis from this study also demonstrated significantly higher transfection efficiencies compared to a static magnet system (11.0 ± 0.7% c.f. 7.9 ± 0.8%, $P < 0.05$) and the lipid reagent (11.0 ± 0.7% c.f. 2.9 ± 0.4%, $P < 0.05$) which uses similar levels of GFP (figure 2E). Although the nucleofection method (Amaxa/Lonza, Tewkesbury, UK) gave higher transfection levels (approximately 20% in our conditions), cell viability was low (5–20%, depending on preset conditions), and the overexpression of GFP decreased over a 72 h period which suggested an overall loss of GFP fluorescence (data not shown). HUVEC transfected with GFP using nTMag yielded

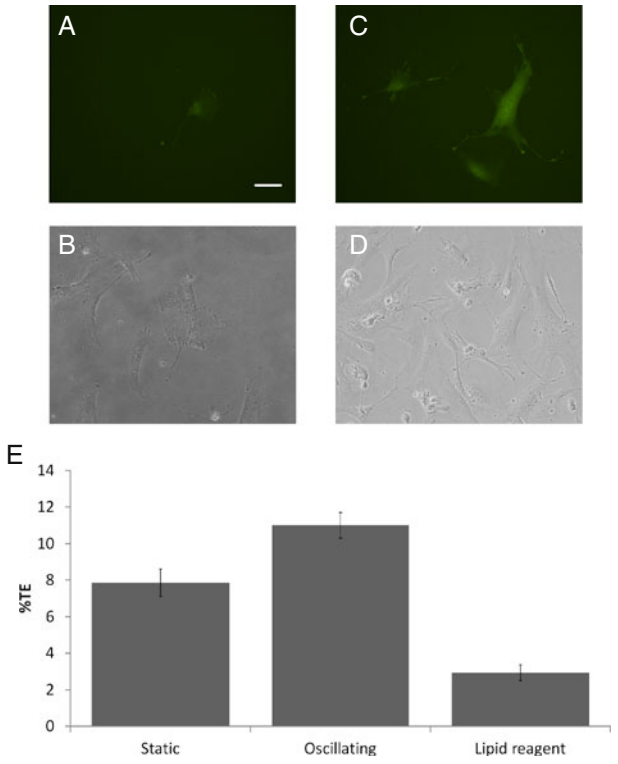

Figure 2. Optimized plasmid transfection efficiency in MEFs. MEFs were transfected with GFP complexes with nTMag as indicated (E, and text), and representative light (B,D) with GFP-fluorescence (A,C) images taken 48 h posttransfection with a low-power objective (10×). Transfection efficiencies (E) for MEF transfected using static (A,B) or oscillating (C,D) (2 Hz amplitude and 0.2 mm displacement) magnetic arrays, and a lipid reagent were determined using flow cytometry and expressed as the mean ± SD of at least three independent experiments. Scale bar: 20 μm.

exceptional results. Like MEF, HUVEC transfected with GFP and nTMag, showed morphologies and densities similar to the untransfected control, as seen under fluorescence microscopy (figure 3, C and D and data not shown). Although HUVEC transfection efficiencies initially were low using both static and oscillating magnet systems, compared with lipofection (7.9 ± 2.3%), transfection efficiency was dramatically improved (figure 3, C&D) by preincubating in serum-free conditions 2 h prior to transfection, at all frequencies including static magnet array, as observed using flow-cytometric analysis (figure 3E). One possible reason could be the dissociation of complexes in the presence of serum which suggests weak binding of plasmid to nanoparticles. However, nTMag nanoparticles showed strong binding to GFP (0.15 μL per 1 μg GFP, data not shown) when compared to another nanoparticle, Neuromag (3.5 μL per 1 μg GFP) (Pickard and Chari 2010). Such difference could be due to particle formulation and concentrations. The use of serum-free conditions to improve transfection was reported in HeLa, KB and H293 cell lines transfected using specific sizes of cationic PEI-polyplexes (Liu *et al.* 2011). This is unsurprising, as this could be due to better DNA incorporation into polyplexes (Liu *et al.* 2011) or onto nTMag particles (this study) in the absence of serum, leading to an increase in number of plasmids per transfected cell upon transfection.

In both serum-containing and serum-free conditions, transfection efficiencies increased with increasing frequencies, with 3 Hz yielding the highest percentage (19.8 ± 0.8%, serum-free) of transfected cells (figure 3E). Frequency-dependent magnet-assisted transfection has been demonstrated earlier and it was suggested that the movements of an oscillating magnet either 'enhance translocation of the particles across the cell membrane' (Kamau *et al.* 2006; McBain *et al.* 2008) or allow DNA release once the complexes are inside the cell.

Finally, overall GFP expression (fluorescence intensity), as reflected from the fluorescence median of the sample, using flow-cytometric analysis, correlates with the transfection levels, with the highest GFP expression seen again at 3 Hz, in serum-free conditions (figure 3F). Together these results demonstrate that the frequency of oscillating magnetic arrays play an important role in the internalization of magnetic nanoparticles complexed with nucleic acids into mammalian cells.

While the transfection of mammalian cells using a static magnetic array has been successfully demonstrated in hard-to-transfect primary cells like MEFs (Seki *et al.* 2006; Lee *et al.* 2011) and HUVEC (Nagata *et al.* 2006), transfection efficiencies were not reported in those publications, perhaps because of the nature of the application involved. A low transfection efficiency of a plasmid might suffice in the case of single-cell time-lapsed imaging (Seki *et al.* 2006). However, if a study involves the overexpression of a gene cloned into a plasmid to overcome an endogenous gene suppressed by a drug/hormone (Nagata *et al.* 2006), a highly effective transfection system will be required to see any significant effect(s). We believe our technology using an oscillating magnetic array with its simplicity, speed, effectiveness and scalability provides a beneficial gene delivery tool for researchers studying cellular function and physiology. The changes of frequency which lead to an increase in transfection, at least in HUVEC, demonstrate that there is much more we could do to exploit this technology (e.g. changing the amplitude of displacement). For example, others working on a static magnet array system, have shown that using a combination of nanoparticles with polyethyleneimine (PEI) can boost transfection efficiency of GFP in HUVEC (Krötz *et al.* 2003). Surely it is a combination of these techniques that will ultimately lead to a breakthrough in the transfection of all hard-to-transfect cell types. Finally this technology can also be used to investigate cellular mechanics and receptor-mediated signalling by observing changes in cells when magnetic nanoparticles bound to the surface of cells come under the influence of an external magnetic field (Dobson 2008). Our technology opens up the field of magnetofection and gene delivery studies

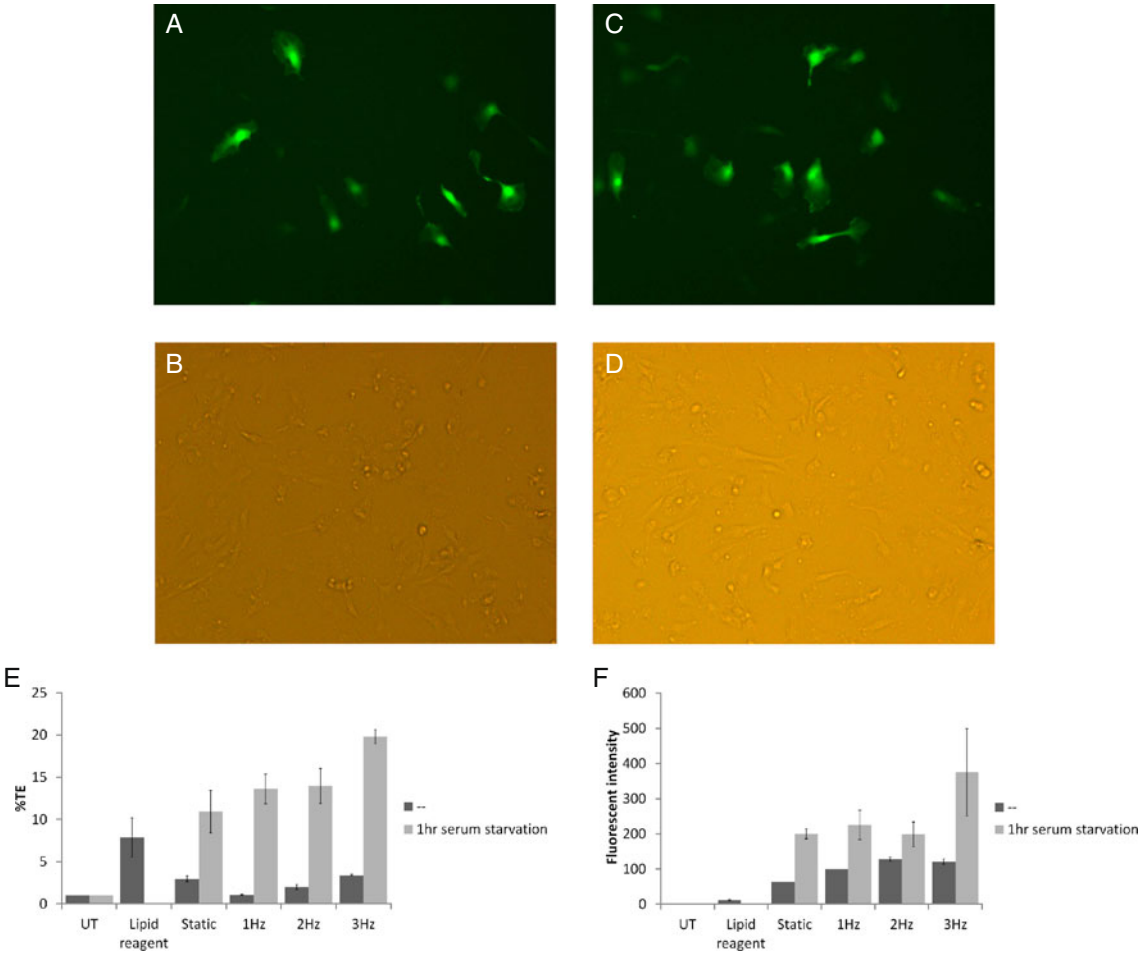

Figure 3. Frequency of oscillation affects plasmid transfection and expression in HUVECs. HUVECs were transfected with GFP complexes with nTMag as indicated (E,F and text), and representative light (B,D) with GFP fluorescence (A,C) images taken 48 h posttransfection with a low-power objective (10×). Transfection efficiencies (E) and fluorescence intensity (median, F) for HUVECs transfected using static (A,B) or oscillating (C,D) magnetic arrays (3 Hz amplitude and 0.2 mm displacement), and a lipid reagent were determined using flow cytometry and expressed as the mean ± SD of at least three independent experiments. Scale bar: 50 μm.

and we expect more exciting publications to emerge in the near future.

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References

Dobson J. 2006 Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery. *Gene Ther.* **13**, 283–287.

Dobson J. 2008 Remote control of cellular behaviour with magnetic nanoparticles. *Nat. Nanotechnol.* **3**, 139–143.

Fouriki A., Farrow N., Clements M. A. and Dobson J. 2010 Evaluation of the magnetic field requirements for magnetic nanoparticle-based gene transfection. *NanoReviews* **1**, 5167.

Jenkins S. I., Pickard M. R., Granger N. and Chari D. M. 2011 Magnetic nanoparticle-mediated gene transfer to oligodendrocyte precursor cell transplant populations is enhanced by magnetofection strategies. *ACS Nano.* **5**, 6527–6538.

Kamau S. W., Hassa P. O., Steitz B., Petri-Fink A., Hofmann H., Hofmann-Amtenbrink H. *et al.* 2006 Enhancement of the efficiency of non-viral gene delivery by application of pulsed magnetic field. *Nucleic Acids Res.* **34**, e40.

Krötz F., Sohn H. Y., Glow T., Plank C. and Pohl U. 2003 Magnetofection potentiates gene delivery to cultured endothelial cells. *J. Vasc. Res.* **40**, 425–434.

Lee C. H., Kim J. H., Lee H. J., Jeon K., Lim H., Choi H. *et al.* 2011 The generation of iPS cells using non-viral magnetic nanoparticle based transfection. *Biomaterials* **32**, 6683–6691.

Liu Z., Zheng M., Meng F. and Zhong Z. 2011 Non-viral gene transfection in vitro using endosomal pH-sensitive reversibly

hydrophobilized polyethylenimine. *Biomaterials* **32**, 9109–9119.

McBain S. C., Griesenbach U., Xenariou S., Keramane A., Batich C. D., Alton E. W. *et al.* 2008 Magnetic nanoparticles as gene delivery agents: enhanced transfection in the presence of oscillating magnet arrays. *Nanotechnology* **19**, 405102.

Muthana M., Scott S. D., Farrow N., Morrow F., Murdoch C., Grubb S. *et al.* 2008 A novel magnetic approach to enhance the efficacy of cell-based gene therapies. *Gene Ther.* **15**, 902–910.

Nagata D., Takahashi M., Sawai K., Tagami T., Usui T., Shimatsu A. *et al.* 2006 Molecular mechanism of the inhibitory effect of aldosterone on endothelial NO synthase activity. *Hypertension* **48**, 165–171.

Pickard M., and Chari D. 2010 Enhancement of magnetic nanoparticle-mediated gene transfer to astrocytes by 'magnetofection': effects of static and oscillating fields. *Nanomedicine (Lond)* **5**, 217–232.

Plank C., Schillinger U., Scherer F., Bergemann C., Rémy J. S., Krötz F. *et al.* 2003 The magnetofection method: using magnetic force to enhance gene delivery. *Biol. Chem.* **384**, 737–747.

Seki T., Irie N., Nakamura K., Sakaue H., Ogawa W., Kasuga M. *et al.* 2006 Fused protein of deltaPKC activation loop and PDK1-interacting fragment (deltaAL-PIF) functions as a pseudosubstrate and an inhibitory molecule for PDK1 when expressed in cells. *Genes Cells* **11**, 1051–1070.

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