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Rotational magnetic pulses enhance the magnetofection efficiency in vitro in adherent and suspension cells

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ABSTRACT

The association of magnetic nanoparticles with gene delivery vectors in combination with the use of gradient magnetic fields (magnetofection) enables improved and synchronised gene delivery to cells. In this paper, we report a system comprising rotating permanent magnets to generate defined magnetic field pulses with frequencies from 2.66 to 133 Hz and a field amplitude of 190 or 310 mT at the location of the cells. Low-frequency pulses of 2.66–10 Hz with a magnetic flux density of 190 mT were applied to the examined cells for 30–120 s after magnetofection. These pulses resulted in a 1.5–1.9-fold enhancement in the transfection efficiency compared with magnetofection with only a static magnetic field in both adherent and suspension cells. The magnetic field amplitudes of 190 and 310 mT had similar effects on the transfection efficacy. No increase in the percentage of transgene-expressing suspension cells and no cytotoxic effects (based on the results of the MTT assay) were observed after applying alternating magnetic fields.

1. Introduction

The delivery of nucleic acids to cells to induce the (over)expression of a desired protein or to down-regulate a target gene has been the focus of increasing attention [1]. Substantial progress has been made in optimising the delivery systems for nucleic acids, and further efforts aim to improve the reliability and effectiveness of cell-modification methods. The feasibility of using engineered adherent and suspension cells, including tumour cells, lymphocytes, dendritic cells [2], fibroblasts, haematopoietic stem cells, and mesenchymal stem cells, for therapy in humans has been demonstrated [3–8]. The applications are as diverse as immunogene therapy for cancer [3,9], the treatment of hereditary diseases [6,10,11], and applications in tissue engineering [12]. Magnetofection™ (see scheme in Fig. 1) is a nanomagnetic method of gene delivery; magnetic micro- or nanoparticles are attached to viral and non-viral nucleic acid delivery vectors, and static gradient magnetic fields are applied to the target site. These methods were first reported in 2000 [13,14], and these reports were followed by publication of the first articles on viral and non-viral magnetofection [15,16]. This technique considerably improves the transfection/transduction efficiency when using low vector doses and allows localised delivery in vitro and in vivo. Magnetofection also saves time and reagents, as reviewed in Ref. [17].

The mechanism of delivery into cells appears to be similar for magnetic and non-magnetic non-viral vectors [18,19]. After non-specifically binding to the cell surface, the complexes are internalised by endocytosis [20–22]. Further escape of the vectors from endosomes, to avoid degradation by the cellular breakdown machinery [23], is thought to be essential for functional nucleic acid delivery and depends on the characteristics of the vector [24,25]. Static gradient magnetic fields accelerate vector sedimentation at the cell surface [26] and can result in a greater internalised vector dose [27–29]. Gradient magnetic fields were also shown to improve the penetration of magnetic vectors in tissues and in a 3D collagen matrix; this improved penetration led to improved cell transfection under the influence of a magnetic field, both in vivo and in 3D model systems [30]. A time-varied magnetic field was also found to enhance the transport of magnetic nanoparticles in a viscous gel [31].

Recently, alternating or pulsating fields were applied to further improve the efficiency of magnetofection [32,33]. A so-called Dynamic Marker magnetic device produces a sinusoidal wave with a maximum amplitude of 27 mT and a field gradient of 10 T/m perpendicular to the well plate. The oscillations of the entire magnetic field perpendicular and parallel to the plate's surface had frequencies of 50 Hz and 0.75 Hz, respectively. The application of a pulsating magnetic field resulted in up to a 2-fold increase on average in the transfection rates relative to the transfection rate for cells exposed only to the static field, depending on the type of cell and the magnetic vector used. The authors speculated that the effect could be attributed to the enhanced cellular uptake

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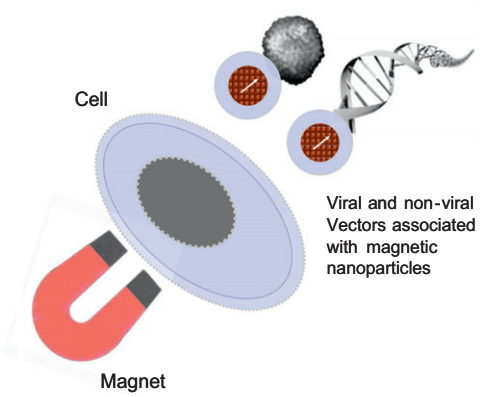

Fig. 1. Principle of magnetofection as illustrated in Ref. [17].

of the complexes bound to the cell surface when alternating fields were applied. The group also reported the improved transfection of primary cells, including synoviocytes, chondrocytes, osteoblasts, melanocytes, macrophages, lung, fibroblasts, and embryonic fibroblasts [34].

Chen et al. have used a different setup with pulsed magnetic fields (0.6 T) of millisecond duration for the rapid transfection of adherent and suspension mammalian cells and *Escherichia coli* [35,36]. The magnetofection efficiency was dependent on the magnetic particle and DNA dose used and on the number of magnetic field pulses; the highest efficiency was achieved by pulsing three times with 2.15 T magnetic fields.

A simple and convenient method to generate alternating fields has been developed by Jon Dobson's group [37], and the system is now commercially available (www.nanotherics.com). In this system, a conventional magnetic plate array producing a static gradient field [16] is rearranged as described [38] and moved below the cell culture plate at a low frequency and a low amplitude. With this set-up, enhancements in the transfection efficiency can be achieved in cell lines and primary cells [37,39,40].

Based on a similar approach, we developed a magnetic system that generates rotational magnetic field pulses with rotating permanent magnets. We tested this system to determine if it could increase the transfection efficiency of model cultured adherent and suspension cells with magnetic delivery vectors. The concept was to use permanent magnets to produce the magnetic pulses without the drawbacks of electromagnets, such as the rapid heating of the system; rapid heating raises the system's resistance and therefore reduces the applied electric current, making it difficult to reproducibly generate defined pulses with electromagnets.

2. Materials and methods

2.1. Magnetic nanoparticles

Core/shell-type iron oxide magnetic nanoparticles (MNPs), hereafter referred to as PEI-Mag2 particles, had an iron oxide core with an average crystallite size of 9 nm; the surface coating was the fluorinated surfactant Zonyl FSA (lithium 3-[2-(perfluoroalkyl)ethylthio] propionate) combined with 25-kDa branched polyethylenimine (PEI-25_{Br}) [29,41]. The hydrodynamic diameter of the particles in aqueous suspension was 28 ± 2 nm. The presence of PEI in the surface layer of the PEI-Mag2 particles resulted in a highly positive net ξ-potential of the particles when measured in aqueous suspension ($+55.0 \pm 0.7$ mV). The saturation magnetisation per unit of iron weight at 298 K was 62 emu/g iron.

SOMag5 core/shell-type magnetic nanoparticles had an iron oxide core with an average crystallite size of 6.8 nm and a silica oxide coating with surface phosphonate groups; the surface phosphonate groups were formed by the co-condensation of tetraethyl orthosilicate and 3-(trihydroxysilyl)propyl methylphosphonate as previously described [42]. The mean hydrodynamic diameter and zeta potential of the MNPs suspended in water were measured to be 40 ± 14 nm and 38.0 ± 2.0 mV, respectively. The saturation magnetisation per unit of iron weight at 298 K was 94 emu/g iron. The aqueous MNP suspensions were sterilised using ^{60}Co gamma-irradiation (25 kGy).

2.2. Cell culture

NCI-H441 human pulmonary epithelial cells derived from a papillary carcinoma of the lungs (ATCC, cat. no. HTB-174), referred to as H441 cells, were cultured in modified RPMI 1640 medium with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g L^{-1} glucose, and 1.5 g L^{-1} sodium bicarbonate supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2.

Jurkat T cells were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, cat no. ACC 282) and maintained at 37 °C and 5% CO_2 in RPMI 1640 medium (Gibco-BRL, Eggenstein, Germany) supplemented with 10% foetal calf serum (FCS), 2 mM D-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (hereafter referred to as complete medium) at a density of 0.75–1.5 million cells/ml. Every 2–3 days, at a density of approximately 1.5 million cells/ml, the cells were split 1:2. The cells were used for transfection experiments until 13–15 passages after thawing.

2.3. Preparation of the magnetic lipoplexes

The luciferase reporter plasmid p55pCMV-IVS-luc+, which contains the firefly luciferase cDNA under the control of the cytomegalovirus (CMV) promoter (pLuc), was amplified and purified by Plasmid Factory, Bielefeld, Germany. The GFP reporter plasmid containing the enhanced green fluorescence protein (peGFP) sequence under the control of the CMV promoter was expanded in *E. coli* and purified using the Qiagen plasmid purification kit.

Magnetic lipoplexes were prepared with eGFP or luciferase plasmid DNA and the DreamFect™ Gold (hereafter referred to as DF-Gold; OZ Biosciences, Marseille, France) as an enhancer at a MNPs/DF-Gold/pDNA ratio of 0.5:4:1 (iron w/v/w). For magnetofection of the adherent cells, PEI-Mag2 MNPs (5 μg Fe in 20 μl of water) were mixed with 40 μl of the DF-Gold, followed by the addition of 40 μl (10 μg) of the pLuc solution in a serum- and supplement-free RPMI 1640 medium. The suspension was kept at room temperature for 20 min to allow complex assembly. Then, the volume was adjusted to 2500 μl with supplement-free RPMI 1640 medium, and 25 μl of the complex solution was added to the 25,000 cells in a well, resulting in a dose of 4 pg plasmid per cell.

For magnetofection of the suspension Jurkat T cells, the SO-Mag5 MNP suspension (25 μg Fe) was mixed with 150 μl of DF-Gold, followed by the addition of 5 μl (25 μg) of the peGFP solution and 5 μl (25 μg) of the pLuc solution in serum- and supplement-free RPMI 1640 medium for a total volume of 500 μl. The DF-Gold/pDNA lipoplexes were prepared using water instead of the MNP suspension. The complexes were applied to the Jurkat cells at a dose of 10 pg of total plasmid per cell. The use of mixed complexes containing both the pLuc and peGFP plasmids allowed for the