The Magnetofection Method: Using Magnetic Force to Enhance Gene Delivery

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In order to enhance and target gene delivery we have previously established a novel method, termed magnetofection, which uses magnetic force acting on gene vectors that are associated with magnetic particles. Here we review the benefits, the mechanism and the potential of the method with regard to overcoming physical limitations to gene delivery. Magnetic particle chemistry and physics are discussed, followed by a detailed presentation of vector formulation and optimization work. While magnetofection does not necessarily improve the overall performance of any given standard gene transfer method in vitro, its major potential lies in the extraordinarily rapid and efficient transfection at low vector doses and the possibility of remotely controlled vector targeting in vivo.

Key words: Gene Delivery / Gene Vectors / Magnetic Drug Targeting / Magnetic Nanoparticles / Magnetofection.

Introduction

Nucleic acid delivery to cells in culture is to a large extent a diffusion-limited process if the nucleic acid shuttle (the vector) is of nanometric size, which is the case for viruses (Chuck et al., 1996) and many synthetic systems. Hence, apart from the major biological barriers including the plasma, endo/lysosomal and nuclear membranes, the limited contact of only a fraction of the applied vector dose with the target cells represents a major limitation to successful and efficient nucleic acid delivery. As a consequence, physical methods that accelerate vector particles toward the target cells have been successful in enhancing nucleic acid delivery. These methods include exploiting gravitational (Luo and Saltzman, 2000) or centrifugal force (Bunnell et al., 1995) which sediment particles on the cells and which are proportional to particle mass. Also convective flow of the transfection medium toward the target cell is a useful tool to enhance gene delivery (Chuck et al., 1996). Furthermore, these methods include biologic methods (the ‘gene gun’ approach; Klein et al., 1992) and the application of electric fields (‘electroporation’, for a review see Somiari et al., 2000). Not surprisingly, one of the earliest nonviral gene delivery methods exploited gravity by the formation of precipitates from DNA and calcium phosphate (Chen and Okayama, 1988). Also with modern synthetic gene transfer systems, large and/or heavy vector particles were more efficient in gene delivery than small particles (Ogris et al., 1998; Luo and Saltzman, 2000). In addition to the mentioned biophysical barriers, also insufficient targetability of nucleic acid vectors to target cells/organs represents a limitation to their use in therapy or as research reagents. In the one extreme, limited targetability is due to the lack of a specific target cell tropism and as a consequence systemic spread of the vector if applied in vivo, limited bioavailability at a desired target site and the possibility of non-specific transfection of non-target cells/organs. In the other extreme, a very specific host tropism can limit the applicability of a vector to a broader target spectrum. This problem prevails for some viral vectors, both in ex- and in vivo gene delivery (Kim et al., 2002). A further limitation can be the rapid inactivation of vectors due to undesired interactions with components of the in vivo milieu before the vector can find its target site. Examples of undesired interactions are opsonization of nonviral vectors (Ogris et al., 1999) or interactions of vectors with host defense systems such as the complement (Plank et al., 1996) or the immune system (Kass-Eisler et al., 1996). Hence, there is an urgent need for simple, affordable and efficient tools for rapid accumulation of gene vectors at their target sites. It would be most desirable if vector accumulation could be remotely controlled by physical means, particularly in the case of intravenous or other systemic administration in vivo.

We have recently developed a method of magnetically
Magnetic Particle Chemistry and Physics

The particles used in such approaches were mostly iron oxides, the synthesis of which is described mainly in the patent literature (see for example Pilgrimm, 1999) but also in text books (Schwertmann and Cornell, 1991). Briefly, such particles are derived by precipitation from acidic iron(II)/iron(III)-sulfate solutions upon addition of bases. Notably, also particles consisting of double oxides/hydroxides of two- or three valent iron with metal ions from the first row of transition metals, such as Co(II), Mn(II), Cu(II), Ni(II) or Cr(III), Gd(III), Dy(III), Sm(III), and furthermore finely milled metallic materials display the characteristics that make them suitable as magnetic carriers. To stabilize such particles they are coated with polymers, for use in biology preferably with biocompatible polymers of synthetic or natural origin. It is important to note that chemists were not the first to design such particles. Magnetite crystals are found in living organisms such as magnetotactic bacteria, in the central nervous systems of fish and even in the human brain (Kirschvink et al., 1992; Merti, 1999; Diebel et al., 2000; Lohmann and Johnsen, 2000). At least for fish, magnetite has been defined as a magnetoreceptor essential for orientation along the earth's magnetic field (Diebel et al., 2000), and there is experimental evidence that similar mechanisms apply in migratory birds (Beason et al., 1995).

The term ‘magnetic particle’ used in the literature and also here generally refers to magnetically responsive solid phases, which are particles or aggregates thereof with micro- to nanometer size. The often used, but physically unprecise term ‘magnetic’, refers to temporarily magnetic materials, which can be ferrimagnetic or ferromagnetic. The term, however, also comprises paramagnetic and superparamagnetic materials. For the precise meaning of these terms the reader is referred to Fahvik et al. (1993) or to text books of physics. The important characteristics for the application described here are that magnetic particles would orient in an externally applied magnetic field, an orientation (magnetization) that however would be immediately overcome by thermal motion upon removal of the external field. In order to have the particles move in a preferred direction of space, they must ‘sense’ an inhomogenous magnetic field (‘gradient field’). The magnetic force acting on such particles in a liquid suspension is proportional to the particle volume, to the magnetic flux density and the magnetic field gradient (Zborowski et al., 1995). This relationship sets the framework that any usage of such particles has to cope with.

Magnetofection – Magnetic Particles in Gene Delivery

The conclusion that what is feasible with low molecular weight compounds should work for nucleic acids as well may appear trivial, however the implementation of such an approach requires a careful consideration of critical
parameters: nucleic acids are charged macromolecules (polyelectrolytes) with their own very peculiar biophysical characteristics (Bloomfield, 1996). Unlike many low molecular weight drugs, they cannot cross the cellular membranes by simple diffusion, and if genes are to be delivered in the form of DNA, the molecules have to reach the nuclei of target cells. Hence, natural transport processes such as endocytosis have to be exploited to deliver these molecules, which holds also true if viruses are used as the nucleic acid shuttle. This means that the association of the vectors with magnetic particles must not interfere with the transport processes used. At the same time, the association has to be stable enough to endure the delivery process itself which in vivo involves interactions with host defense mechanisms and a multitude of solutes in blood and other body fluids. All this imposes certain requirements on the physical nature (size and surface characteristics) of the transport system as a whole, but also of its components, and also on the linkages that hold the components together. Provided that magnetic particles with appropriate reactive surface groups are available, physical, chemical and biological linkages can be envisaged. Among the latter, biotin-streptavidin and antigen-antibody bridges have been successfully used to link viruses to nonviral vector particles (Curiel et al., 1991; Wagner et al., 1992) and more recently also to link viruses to magnetic particles (Hughes et al., 2001; Mah et al., 2002; Pandori et al., 2002). The term nonviral vector particle refers to complexes of DNA with polycations (‘polyplexes’) or cationic lipids (‘lipoplexes’). These complexes have been shown by numerous researchers to be particles of nanometric size or aggregates thereof. Often, toroids and rods are observed for polyplexes and less regular structures for lipoplexes, sometimes extending into the micrometer size range (an example of such structures is shown in Finsinger et al., 2000). As (strept)avidin or antibody-coated magnetic particles are expensive to manufacture, we decided to use the simplest way of linkage, physical interaction, which not only is the cheapest way but also highly effective and fully compatible with the cellular processing of gene vectors. Therefore we used magnetic particles coated with polyelectrolytes (in the following often referred to as ‘magnetic particles’) which are, in the same manner as many nonviral vector particles, subject to salt-induced aggregation (Figure 2A), a phenomenon well known in colloid science (Hiemenz, 1986). In this manner, simple mixing of polyelectrolyte-coated magnetic particles and gene vectors at an appropriate ratio in salt-containing medium was sufficient to achieve quantitative association (Scherer et al., 2002). It is important to note that when pre-assembled nonviral vectors are associated with polyelectrolyte-coated magnetic particles, the vector and the magnetic particles do not need to have opposite surface charges. In fact, in

Fig. 2 Vector Association with Magnetic Particles: Salt-Induced Colloid Aggregation.
(A) A ternary complex was prepared in water by mixing DNA with PEI-coated magnetic particles and additional free PEI (10 µg DNA/ml final concentration; 25 kDa PEI from Aldrich). Salt-induced aggregation was initialized by adjusting the ionic strength to 150 mM sodium chloride. The complex aggregated with approx. linear kinetics starting at 217.0±2.0 nm and remaining in the sub-micrometer range within two hours (size measurement by dynamic light scattering using a Malvern 3000 HS Zetasizer). (B) Electron micrographs of PEI-coated magnetic particles (transMAG-PEI, left) and their associates with PEI-DNA vectors produced by salt-induced aggregation (right) as described in (A). The micrographs show that the magnetic particles are multi-domain structures of irregular shape. The resulting DNA complexes (right) display a tight association of PEI-DNA structures (gray/black spherical structures) with magnetic particles. Electron microscopy was performed as described by Erbacher et al. (1999). (C) DNA binding isotherms. DNA was labeled with [125I] according to the Commerford method modified as described by Terebesi et al. (1998). DNA (squares) or PEI-DNA complexes (circles; N/P ratio=8) were mixed with magnetic particles in water at increasing particle:DNA weight ratios, final DNA concentration 10 µg/ml. The N/P ratio is the ratio of the nitrogen atoms of PEI to the phosphate groups of DNA in the complex. In water (open squares and circles) magnetic particles did not associate with PEI-DNA complexes but bound naked DNA. Upon adjustment of the ionic strength to 150 mM sodium chloride, binding of naked DNA was enhanced up to 1.7-fold at a given weight ratio. A sigmoidal binding curve was obtained for PEI-DNA. The binding curves demonstrate the importance of salt-induced aggregation for vector-particle association and show that positively charged magnetic particles can easily be associated with positively charged gene vectors. Interestingly, PEI-DNA vectors displayed a similar binding behaviour with negatively charged magnetic particles (data not shown).
most of our experiments we associated positively charged vector particles with positively charged magnetic particles (Figure 2). The appropriate ratios of magnetic particle and vector (or naked DNA) were found by serial titrations of radiolabeled DNA with magnetic particles followed by magnetic sedimentation and determination of the DNA fraction remaining in the supernatants (Figure 2B). The binding data were then set in correlation to gene transfer efficiency data obtained with multi-dimen-sional titrations of vector dose/magnetic-particle-to-DNA ratios/vector type in various standard cell lines. From such titrations we found numerous examples where magnetofection was clearly superior to standard trans-

![Graph A: 10 min, with magnet](image)

![Graph B: 10 min, without magnet](image)

![Graph C: 4 h, with magnet](image)

![Graph D: 4 h, without magnet](image)

**Fig. 3** Dose-Response Profiles of Magnetofection of CHO-K1 Cells with the GenePorter Reagent.

One vol. equivalents of DNA stock solutions in DMEM (20 µg/ml) were added to equal volumes each of a serial dilution of magnetic particles in DMEM, mixed, and then mixed immediately with two vol. equivalents GenePorter dilution (50 µl/ml in DMEM). The samples were diluted with DMEM to a volume corresponding to 20 vol. equivalents, then a series of two-fold dilution steps in DMEM was performed in a 96-well plate. The medium from cells that were seeded in 4 plates at a density of 20 000 cells/96 well was removed and 100 µl each of DNA complexes were added in triplicates. Two plates each were incubated as usual and two plates each on a magnetic plate for 10 min and then supplemented with fresh medium containing 10% FCS (+ pen/strep) were added in triplicates. Two plates each were incubated as usual and two plates each on a magnetic plate for 10 min and 4 hours, respectively. The 10 min incubation plates were washed and then supplemented with fresh medium containing 10% FCS and penicillin/streptomycin. To the 4 hour plates 100 µl/well of fresh medium containing 20% FCS (+ pen/strep) were added without a washing step. The luciferase assay was carried out as described (Finsinger et al., 2000) after 24 hours. The Figure shows that the transfection efficiency decreased at higher magnetic particle doses probably due to toxicity. A magnetic particle to DNA ratio of 4 turned out useful at all DNA doses. The data points represent the averages of triplicates ± standard deviations.

It was an interesting finding that the mixing order of the vector components (DNA, polyelectrolyte-coated magnetic particles, third components such as cationic lipids or polyanion (an example is shown with the lipofection reagent DOTAP-cholesterol in Figure 4). Also, the transfection efficiency was largely independent of the type of polycation or polyanion used for particle coating (data not shown). Various coatings including PEI of various molecular weights, DEAE dextran, poly(aspartic acid), poly(maleic acid), poly(acrylic acid), or phosphate-derivatized starch were tested. This formulation work led us
equivalent DNA stock solution in HBS (30 µg/ml) was mixed with
tion time of 15 to 30 min, the magnetic vectors are added
to the cells in standard culture plates which are posi-
tions from a number of suppliers (including Dexter, Promega, Miltenyi, Dynal) and may be useful for mag-
fed vectors on the cells. On the other
hand, an appropriate positioning of magnets, it is
easy to obtain a selective transfection of only part of a
cell population within one well (Pandori et al., 2002; Scherer et al., 2002). While this already demonstrates the principle possibility of selective magnetic field-directed targeting for in vivo gene delivery, this feature may also be a useful technical tool for evaluating gene or protein func-
tion having the untransfected cell control within the same
well.

Mechanism and Benefits of Magnetofection

The major efficacy-enhancing mechanism of magneto-
fection appears to be the rapid sedimentation of the full
vector dose on the target cells, such that up to 100% of
these cells will have vector particles bound to their
surfaces within a few minutes (see for example Figure 9).
Achieving the same frequency of vector-target cell con-
tact may take hours during conventional transfection.
Nevertheless, an additional effect of the magnetic field
can not be excluded. It has been shown that upon injec-
tion of magnetic particles into rat tail veins and position-
ing of a strong permanent magnet downstream of the
jection site, magnetic particles are found outside the
blood vessel, implying that the magnetic field had caused
extravasation into the surrounding tissue (Gupta and
Hung, 1993). Therefore, it can be speculated that the
magnetic field might pull the magnetic particle-vector as-
nociates across the plasma membrane into the cells.
Also, as of yet unknown changes in cellular physiology
upon exposition to the magnetic field may play a role.
However, our results indicate that the primary uptake
mechanism proceeds via the endo/lysosomal pathway,
as we have argued before (Scherer et al., 2002). With
naked DNA bound by electrostatic interaction to polye-
cation-coated magnetic particles we only achieve low
efficiency gene transfer. When to this associate PEI,
cationic lipids, a membrane destabilizing agent such as
the peptide INF7 (Plank et al., 1994) or an inactivated
adenovirus (Cotten et al., 1994) are added, gene delivery
is greatly enhanced. All these agents have been inferred
to mediate endo/lysosomal release upon endocytotic
vector uptake and therefore to enhance the transfection
eficiency. Another strong evidence of endosomal uptake
comes from electron microscopic studies that we have
carried out (Figure 5). We found magnetic particles asso-
ciated with a majority of the cells within 15 min of mag-
netic field exposure. We found particles in cellular invagi-
nations resembling coated pits (Figure 5A). After 24 hours
we often found the particles in membrane-surrounded
structures which may be endosomes or lysosomes (Fig-
ure 5B). When the DNA component was labeled with col-
loidal gold, we found co-localization of magnetic parti-

Fig. 4 Magnetofection of NIH 3T3 Cells Using the DOTAP-
Cholesterol Reagent and Magnetic Particles with Positive and
Negative Surface Charges.

DOPA (1,2-dioleoyl-3-trimethylammoniumpropane) was pur-
chased from Avanti Polar Lipids (Alabaster, AL, USA). DOTAP/
cholesterol (molar ratio 1:0.9) liposomes were prepared essential-
ly as described (Meyer et al., 1995) as 5 mM (DOTAP) suspension
in 5% glucose. Two mixing protocols were used. [ ]: one vol.
equivalent DNA stock solution in HBS (30 µg/ml) was mixed with
one vol. equiv. DOTAP/chol liposome stock (455 µM DOTAP in
HBS) followed by mixing with one vol. equiv. magnetic particle
suspensions (30 µg/ml). [ ]; using the same reagents, first
magnetic particles and DNA were mixed and then added to
DOTAP-chol. Magnetic particles were transMAG-PO4 (coating
with phosphorylated starch), transMAG-PEI (coating with PEI). A
dilution series was prepared to yield the indicated DNA doses per
well (50 µl aliquots of DNA complexes were added per well in trip-
licates). Cells were seeded 7 hours prior to transfection at a density
of 50 000 cells/well in 150 µl DMEM/10% FCS/pen,strep. Per set-
up, experiments were carried out with (full symbols) and without
positioning on a magnetic plate (open symbols) during 10 min of
incubation followed by medium change.

to a simple protocol of magnetic vector formulation and
application: the gene vector is prepared as usual with
commercially available reagents according to the instruc-
tions of the manufacturer. In an additional step, the pre-
formed vector is then mixed with an amount of magnetic
particles such that the weight/weight ratio of magnetic
particles and DNA will be 1:1 and higher. After an incuba-
tion time of 15 to 30 min, the magnetic vectors are added
to the cells in standard culture plates which are posi-
tioned on a magnetic plate (Scherer et al., 2002) for
15 minutes. After this time, the medium can either be
changed or left on the cells until further evaluation (such
as luciferase assay etc.). Magnetic plates for transfection
purposes are commercially available (chemicell, Berlin,
Germany) but can also be home-made with strong per-
manent magnets (Scherer et al., 2002). Similar plates are
available mostly for cell or nucleic acid separation pur-
poses from a number of suppliers (including Dexter,
Promega, Miltenyi, Dynal) and may be useful for mag-
fetfection, depending on the geometry of the magnet
positioning. The essential requirement for magnetofec-
tion is that the magnetic particle-associated vectors ex-
perience a magnetic gradient field strong enough to pull
tor displayed a linear relationship between luciferase expression and incubation time of cells with the vector cocktail. The same experiment carried out with Lipofectamine (Figure 6A and C) resulted in even decreasing transfection efficiency with increasing incubation time in the case of magnetofection, while the standard vector displayed a minor linear increase but being rather inefficient overall. Taken together, the transfection kinetics is another indication that the major enhancing factor in magnetofection is the rapid accumulation of the full vector dose at the target cell surface. This also led to a very early onset of reporter gene expression (as shown in Figure 7 for PEI-DNA complexes). Within 2 hours after a 15 minute magnetofection period, significant reporter gene expression levels were detectable, while it took between 4 and 8 hours to reach the minimal detectable expression level by standard transfection. For some vectors, another beneficial consequence of the rapid cell binding of the full vector dose was the highly favorable dose-response profile of magnetofection compared to standard transfection. For example, logarithmic profiles were observed for magnetofection with PEI, DOTAP-Cholesterol or Lipofectamine in contrast to a linear profile for standard transfection (Figure 4, DOTAP-Cholesterol; Figure 6, Lipofectamine). This means that by applying magnetofection, high transgene expression levels can be achieved at a very low vector dose with extremely short incubation times. This allows avoiding toxicity with cells that are sensitive to the transfection process (Krötz et al., 2003). High transgene expression levels are either achieved by having a few cells expressing tremendous amounts of the transfected gene or by having a high percentage of target cells transfected. In most of our magnetofection experiments we observed a combination of both, a high percentage of transfected cells producing high amounts of transgene product (as shown in Figure 8 for CT26 colon carcinoma cells transfected with DMRIE-C and as also shown in Scherer et al., 2002, for adenoviral and retroviral magnetofection). Despite this success it needs to be emphasized that the final transgene expression level achieved with magnetofection is not necessarily higher in all cases than the one achieved under optimized conditions with a given standard transfection protocol (as is also evident from Figures 3 and 6). Like with all gene delivery methods, this is dependent on the cells to be transfigured and on the skills and the awareness of the experimentator. In summary, the benefits of magnetofection are that (i) it saves time, (ii) it saves material, (iii) it can potentiate the efficacy of a given vector, and (iv) it allows magnetic field-guided targeting.

Applications of Magnetofection

These features make magnetofection a very useful tool where any one or several of these key points limit the success of a given approach involving nucleic acid delivery. There are multiple examples of such technical challenges: from gene therapy, where nucleic acid delivery i-
The self is at the heart of the therapeutic strategy, to basic research or even commercial scale biotechnology approaches where nucleic acid delivery only serves as a tool for answering scientific questions. An example for the latter is a particular functional genomics approach where known or unknown DNA or RNA sequences are transfected into cells in a high throughput manner in order to obtain novel screenable phenotypes (see for example the experiment described in Figure 3 with a fixed magnetic particle:DNA ratio of 2:1 (w/w), a DNA dose of 100 ng/well for the kinetics study in (A) and (B), and a serial dilution for the dose-response study in (C) and (D), and with 4 µl of Lipofectamine and 5 µl of GenePorter used per µg of DNA, respectively. The experiments shown in (A) and (B) were carried out with one culture plate each positioned upon the magnetic plate throughout the maximum incubation time of 240 minutes, one plate was kept under standard conditions. The vector formulations were removed from individual wells in triplicates each and cells in these wells were washed after 5, 10, 20, 40, and 240 minutes of incubation. The luciferase assay was carried out 20 hours after the start of the experiment. Under the conditions tested, maximum expression was found already after 5 min with Lipofectamine, while transfection efficiency increased over time but with a moderate slope with GenePorter (42% of the final reporter gene expression level was already achieved after 5 min). Consistently, the Figures show that 10 min of magnetofection are sufficient to achieve the transfection levels obtained with the standard reagent using 4 hours of transfection. At any time point, GenePorter formulations were more efficient than Lipofectamine formulations.

**Fig. 6** Transfection Kinetics and Dose-Response Profile of Standard Transfection and Magnetofection in NIH 3T3 Cells Using the Reagents Lipofectamine and GenePorter.

**Fig. 7** Reporter Gene Expression Kinetics of Magnetofected or Standard PEI-Transfected Luciferase Plasmid.

One vol. equivalent of a DNA stock solution (60 µg/ml) was mixed with one vol. equivalent of a PEI stock solution (62.6 µg/ml, 25 kDa branched PEI, Aldrich) or one vol. equivalent of a magnetic particle suspension (240 µg/ml), respectively (all dilutions in 0.9% sodium chloride). After 15 min of incubation one vol. equiv. of 0.9% sodium chloride was added to the PEI-DNA complexes and one vol. equiv. of PEI stock solution was added to the magnetic particle-DNA complexes. After 15 min of incubation, 50 µl each (DNA dose 1 µg) of the resulting complexes were added to the cells seeded in two 96-well plates at a density of 20 000 cells/well, covered with 150 µl fresh medium (DMEM, 10% FCS). One plate was positioned on the magnetic plate throughout the first 8 hours of incubation, after which a medium change was performed (DMEM, 10% FCS). During this incubation time, cells in triplicate wells each were washed with PBS and lysed for luciferase assay after 1, 2, 4, and 8 hours of incubation and then again after 24 and 48 hours. The luciferase assay was performed as described (Finsinger et al., 2000). The figure shows the early onset of detectable reporter gene expression upon magnetofection which peaked after 24 hours and which was consistently higher than the one achieved with standard transfection (enhancement of 9- to 18-fold after onset of reporter gene expression in the standard wells).
ample Grimm et al., 2002). On the one hand this would allow to assign function to a given nucleic acid sequence, on the other hand it would allow to screen a given nucleic acid pool for therapeutically useful sequences, and in a further step to optimize such sequences in terms of a desired biological effect. Whatever the goal of such an approach may be, it would require thousands of successful transfections a day carried out in an automated manner. Process time, material consumption (and connected costs) and transfection efficiency represent the key bottle necks to such strategies. It can easily be envisaged to incorporate the magnetofection technology in a robotic system which would provide the solution to this technical challenge.

Our own long-term goal is the use of magnetofection as a tool for gene therapy, on the one hand for ex vivo approaches potentially useful in tissue engineering or for the generation of tumor vaccines, on the other hand for localized gene delivery in vivo with potential applications in cardio-vascular or, again, cancer therapy. Any of these purposes would involve the transfection of primary cells with the major technical challenges of efficacy and of targeting. Consequently, we have recently focussed our attention on establishing the technique in primary cells with reporter genes. We have shown that the association of adenovirus with magnetic particles provides the virus with the ability to infect cells which are non-permissive to adenoviral gene delivery because they lack the required virus receptor (CAR) (Scherer et al., 2002). This is important because the absence of this receptor in natural targets of adenoviral gene therapy, such as tumors, severely limits the feasibility of conceptionally highly promising approaches (Kim et al., 2002). Similarly, we have shown localized adenoviral and nonviral gene delivery by magnetofection in vivo in blood vessels and in the gastro-intestinal tract (Scherer et al., 2002). Our most recent results show that nonviral magnetofection is highly efficient in the ex vivo transfection of primary cells including hu-

**Fig. 8** Magnetofection and Standard Transfection of the Colon Carcinoma Cell Line CT26 with the Transfection Reagent DMRIE-C Using Green Fluorescent Protein Gene as Reporter. One vol. equiv. DNA stock (12 µg/ml in Optimem) was mixed with 2 vol. equiv. DMRIE-C suspension (30 µl/ml in Optimem), followed by mixing with one vol. equiv. magnetic particles (12 µg/ml in Optimem) or with Optimem alone. A dilution series was prepared after 30 min of incubation. Cells (seeded in 24 well plates, supplemented with 200 µl/well fresh Optimem prior transfection) were transfected by addition of 200 µl of complexes, containing DNA doses of 600, 300 and 150 ng DNA. The culture plate was positioned on a magnetic plate during the first 15 min of incubation, which was continued for a total of 5 hours. Then the medium was exchanged for RPMI1640 medium containing 10% FCS. Fluorescence images were taken 24 hours after complex addition. The Figure shows the strong improvement in percentage of transfected cells which is particularly apparent at the lower DNA doses. Magnetofection of 150 ng DNA resulted in higher efficiency than with 600 ng DNA in standard vector formulation.

**Fig. 9** Magnetofection and Standard Transfection of Anti-sense Oligonucleotides in HUVECs. Cy3-labeled anti-SHP-1 phosphorothioate oligodeoxynucleotides (ODN) were transfected using Effectene™ in combination with magnetofection. Antisense-ODN (200 ng per well of a 24-well plate, ca. 60 nM) were mixed with buffer EC (volume added to result in a final volume of 30 µl after step (1), Effectene™ enhancer solution (w/v ratio of 8) and magnetic particles (B) (1 mg/ml suspension; w/w ratio of 1) or water only (A), vortexed for 1 second, and incubated for 2–5 minutes (step 1). Then, Effectene™ (w/v ratio of 20) was added, followed by vortexing for 10 seconds and incubation for 5–10 minutes, and terminated by addition of medium 199 supplemented with 20% NBCS and antibiotics (HUVEC medium) to a final volume of 200 µl (transfection mixture). Before addition of the transfection mixture (200 µl each) to confluent HUVEC, the cells were washed once with phosphate buffered saline (PBS) and supplemented with 200 µl of HUVEC medium. The culture plates were positioned on magnetic plates for 15 minutes, followed by washing the cells 3 times with PBS and continued cultivation in medium for 2 hours. Images were taken using an inverted laser scanning microscope (LSM 410, Zeiss, Jena, Germany). (A) Standard transfection; (B) magneto-
man umbilical vein endothelial cells (HUVEC), porcine aortic endothelial cells (PAEC) (Krötz et al., unpublished results), rat carotid artery smooth muscle cells, explanted porcine veins, porcine and rabbit articular chondrocytes, human and porcine airway epithelial cells, intact porcine tracheal airway epithelium, and human keratinocytes (unpublished results). We were also successful in applying magnetic particle–DNA complexes as aerosol directly on human airway epithelial cells in culture with subsequent efficient transfection by magnetic field influence, giving a novel perspective to gene delivery to the lungs via the airways (J. Rosennecker, unpublished results).

We have used magnetofection with great success not only for the delivery of viruses and plasmid DNA but also for the delivery of antisense oligonucleotides (Krötz et al., 2003). The goal of this study was to define the role of the p22phox subunit of endothelial NAD(P)H-oxidase in superoxide production in primary endothelial cells by an antisense knock-out approach. Measuring such a sensitive cellular function required that the oligonucleotide was delivered at low dose, rapidly, and that a high percentage of the target cells (HUVEC) were transfected. We found that by magnetofection, oligonucleotides, applied at low dose, became associated within minutes with up to 100% of the target cells (Figure 9), and were then rapidly internalized and accumulated in the nuclei. This resulted in the efficient knock-out of the target which then allowed us to demonstrate the important role of the p22phox subunit of endothelial NAD(P)H-oxidase in the enzyme’s basal activity. Conventional transfection was associated with significant toxicity as it either required a high vector dose and/or long incubation times (24 hours) to yield the required efficiency. However, when measuring sensitive physiological functions such as superoxide production, the scientific question itself is incompatible with transfection-associated toxicity as it would have a direct impact on the cellular function to be measured. In the same study (Krötz et al., 2003) we demonstrated the general applicability of antisense magnetofection with two additional independent targets of antisense knock-out (connexin 37 and cytosolic phosphatase SHP-1; data not shown here). Finally, we also demonstrated the feasibility of magnetic field-guided oligonucleotide targeting to blood vessels in vivo which in the future will allow the examination of gene function directly in vivo with having the untreated control blood vessels in the same animal (data not shown).

Conclusions and Outlook

During the last fifteen years we have witnessed the development of highly efficient tools for gene delivery to eukaryotic cells and more recently also their successful use in gene therapy (Aiuti et al., 2002a,b; Fischer et al., 2002). Despite these impressive achievements it may take additional decades until gene therapy becomes a broadly applicable therapeutic modality, particularly considering the many unknown factors and risks associated with any novel form of treatment. But this apparent delay with respect to original expectations is also due to unmet technical requirements, among other things relating to gene vector efficacy, safety and economic feasibility of its production and handling. Although the technique of magnetofection is far from providing a single general solution to these deficiencies, it opens a novel perspective on this stony path. First of all, it provides a technical solution for the rapid and efficient introduction of nucleic acids into primary cells which will greatly facilitate and accelerate the examination of gene function, the identification of nucleic acids with therapeutic potential and also the assessment of risks associated with the transfer of genetic material into cells. Secondly, the technique can become an option for in vivo targeting combined with improved efficacy of gene delivery, potentially in combination with other physical techniques, if appropriate magnetic fields can be generated. As mentioned before, the essential physical parameters governing the force acting on magnetic particles in liquid suspension are the magnetic flux density, the magnetic field gradient and the particle volume. Optimizing these parameters for biomedical applications raises several demanding engineering challenges in this exciting field of interdisciplinary research.

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