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REVIEW

Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery

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The recent emphasis on the development of non-viral transfection agents for gene delivery has led to new physics and chemistry-based techniques, which take advantage of charge interactions and energetic processes. One of these techniques which shows much promise for both in vitro *and* in vivo *transfection involves the use of biocompatible magnetic nanoparticles for gene delivery. In these systems, therapeutic or reporter genes are attached to magnetic nanoparticles, which are then focused to the target site/cells via high-field/high-gradient magnets. The technique promotes rapid transfection and, as more recent work indicates, excellent overall transfection levels as well. The advantages and difficulties associated with magnetic nanoparticle-based transfection will be discussed as will the underlying physical principles, recent studies and potential future applications.*
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In brief

Progress

- Theoretical considerations suggest key variables for augmenting gene transfer.
- The development of new magnetic nanoparticles is leading to improvements in transfection efficiency.
- The recent refinement of magnetofection techniques demonstrates that it significantly reduces transfection time in comparison to other non-viral agents and has been used to successfully deliver small-interfering RNA and antisense oligonucleotides *in vitro* and *in vivo*.
- Oscillating magnet arrays show promise for further enhancing magnetic nanoparticle-mediated gene delivery.

Prospects

- The use of carbon nanotubes also shows great promise; however, the potential for *in vivo* use may be more limited in the near-term owing to the potential for toxicity.
- The use of oscillating arrays of permanent magnets has been shown to significantly increase overall transfection levels even well beyond those achievable with cationic lipid agents.
- The continued development of new particles and new magnetic field application techniques will lead to further rapid advances in magnetofection technology both *in vitro* and *in vivo*.

Introduction

Magnetic nanoparticle-based transfection methods are based on the principles developed in the late 1970s by Widder and others for magnetically targeted drug delivery. The use of magnetic microparticles for transfection was first demonstrated in 2000 by Cathryn Mah, Barry Byrne and others at the University of Florida, *in vitro* in C12S cells and *in vivo* in mice using an adeno-associated virus (AAV) linked to magnetic microspheres via heparin. Since these initial studies, the efficiency of this technique, often termed 'magnetofection', has been demonstrated in a variety of cells.

The technique is based on the coupling of genetic material to magnetic nano- (and in some cases, micro-) particles. In the case of *in vitro* magnetic nanoparticle-based transfection, the particle/DNA complex (normally in suspension) is introduced into the cell culture where the field gradient produced by rare earth magnets (or electromagnets) placed below the cell culture increases sedimentation of the complex and increases the speed of transfection (Figure 1).

In vivo, magnetic fields focused over the target site have the potential to not only enhance transfection but also target the therapeutic gene to a specific organ or site within the body (Figure 2). Generally, particles carrying the therapeutic gene are injected intravenously and strong, high-gradient external magnets are used to capture the particles as they flow through the blood-

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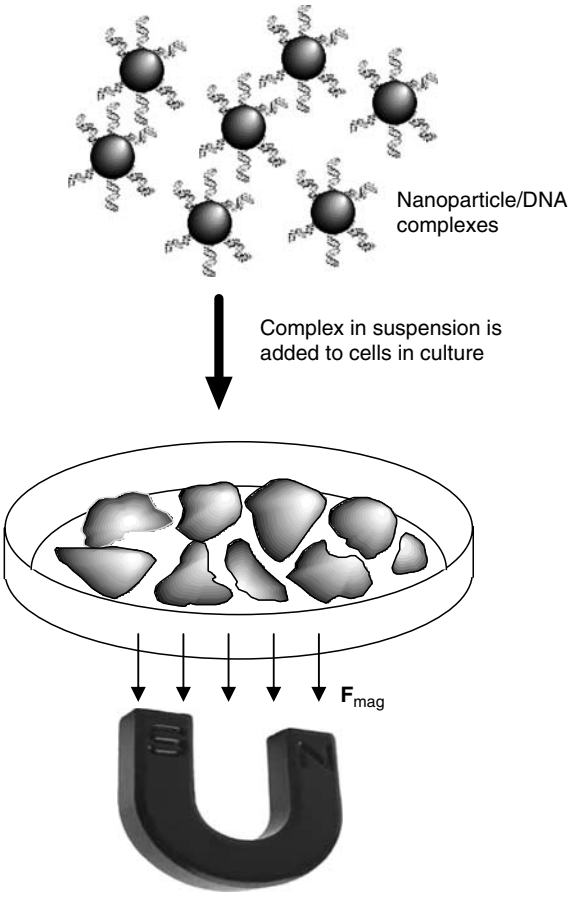

Figure 1 Schematic representation of magnetic nanoparticle-mediated gene delivery *in vitro*. The vector is attached to magnetic nanoparticles, which are added to the cell culture. A high-gradient, rare-earth magnet is placed below the culture dish and the magnetic field gradient pulls the particles towards the magnetic field source, increasing the sedimentation rate of the particle/gene complex. F_{mag} is the force vector exerted on the particles by the magnetic field.

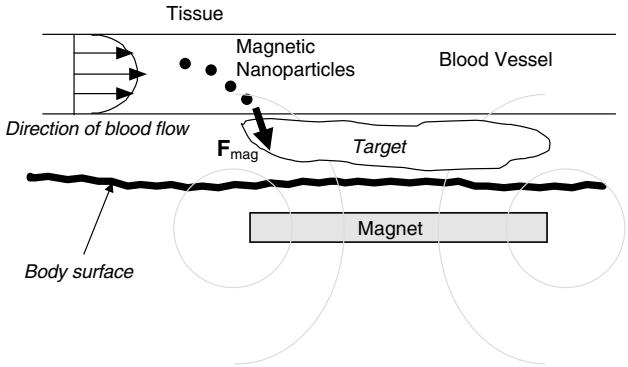

Figure 2 Schematic representation (side view section) of magnetic nanoparticle-based gene targeting *in vivo*. Dashed gray rings indicate the lines of magnetic flux due to the *ex vivo* permanent disc magnet. F_{mag} is the magnetic force vector exerted on the particles as they flow through the bloodstream (Figure redrawn after Pankhurst *et al.*, 2003).

stream. Once captured by the field, the particles are held at the target, where they are taken up by the tissue. The therapeutic genes can be released either via enzymatic cleavage of the cross-linking molecules, charge interactions, or degradation of the polymer matrix. Alternatively, if the DNA is embedded within the matrix, such as with hydrogels, alternating fields may be applied to heat the particles and release the genes from the magnetic carrier.

Theoretical considerations suggest key variables for augmenting gene transfer

The physical principles of magnetofection are virtually the same as those underlying magnetic nanoparticle-based drug targeting. This technique is based on the attractive force exerted on magnetic particles by a magnetic field source according to the equation:

$$F_{\mathrm{mag}} = (\chi_2 - \chi_1)V\frac{1}{\mu_{\mathrm{o}}}B(\nabla B)$$

where F_{mag} is the force on the magnetic particle, χ_2 is the volume magnetic susceptibility of the magnetic particle, χ_1 is the volume magnetic susceptibility of the surrounding medium, μ_o is the magnetic permeability of free space, V is particle volume, B is the magnetic flux density in Tesla (T), ∇B is field gradient and can be reduced to $\partial B/\partial x$, $\partial B/\partial y$, $\partial B/\partial z$.[1] It is clear from this equation that in order to generate a force on the magnetic particle, the magnetic field must have a gradient. In the presence of a homogeneous field, the particle will experience no force. For this reason, high-gradient, rare-earth magnets are commonly used for both magnetic nanoparticle-based drug delivery as well as for magnetofection applications. This equation also indicates that the variable parameters that can be used to increase the force (and, *in vivo*, the likelihood of capture) on the magnetic particle carrying the therapeutic or reporter gene are the particle volume (larger particles = more force), magnetic field strength, magnetic field gradient and the magnetic properties (suspectibility) of the particles.

It was only recently, however, that the theoretical aspects of magnetic targeting had been examined in detail using physiologically relevant models.[2] Earlier theoretical work by Ruuge, Volairis and others indicated that for most magnetic carriers, the magnetic flux density (field strength) at the target site must be of the order of 200–700 millitesla (mT) in order to efficiently capture particles flowing in the blood vessels. In addition to this high field strength, field gradients along the z-axis of 8–100 T/m are required, depending on the blood flow rate at the target. These results gave a preliminary indication that magnetic nanoparticle-based targeting was likely to be more effective for target sites that are close to the surface of the body and/or in regions of relatively slow blood flow. The models used to derive these parameters, however, were rather simplistic in many of their assumptions.

As mentioned, a more recent mathematical model by Grief and Richardson[2] has been developed, which is more realistic and examines a vareity of field/particle configurations in a two-dimensional branching network of blood vessels. This model incorporates sheer-induced diffusion due to the presence of cells within the blood plasma – a factor that was neglected in earlier models. This new model demonstrates that it will be difficult to target a specific site at depth within the body without

some degree of distribution to the intervening tissue. Thus, the tissue between the target and the magnet source (which is outside the body) will be more strongly affected by the field as it is closer to the field source. For these reasons, Grief and Richardson also conclude that magnetic nanoparticle-based drug/gene delivery is likely to be most effective for target sites near the surface of the body, close to the source of the magnetic field.

The development of new magnetic nanoparticles is leading to improvements in transfection efficiency

In the case of magnetofection, as in the case of magnetic drug delivery, the gene is attached directly to the magnetic particle or carrier. These particles generally consist of a magnetic iron-oxide either dispersed within a polymer matrix – such as silica, polyvinyl alcohol (PVA) or dextran – or encapsulated within a polymer or metallic shell (e.g., Neuberger *et al*.[3] and Harris *et al*.[4]). The shell or matrix can be functionalized by attaching carboxyl groups, amines, biotin, streptavidin, antibodies, etc. In the case of *in vitro* magnetofection, the particles are usually coated with polyethyleneimine (PEI), which binds DNA to the particle's surface via charge interactions.

Work by our group at Keele has focused on the development of mesoporus silica nanoparticles which can have up to 80% iron oxide content.[5,6] These particles are being functionalized with a variety of molecules to promote uptake by the target cells and have also been associated with *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTAP chloride), which appears to prevent aggregation and promote uptake.

In a recent work by Cai *et al*.,[7] carbon nanotubes were shown to be exceptionally promising as non-viral gene delivery agents. The group has pioneered a technique they call 'nanotube spearing'. The basic principles involve the preparation of nickel-embeded, magnetic nanotubes to which DNA is attached. These nanotubes are elongated and, as such, when exposed to a magnetic field, they align parallel to the lines of magnetic flux, in a similar fashion to the way iron filings line up when sprinkled above a bar magnet. The presence of a gradient field as well as the angle between the magnetic flux lines and the cell membrane, cause the particles to move toward the membrane with an orientation similar to that of a spear being thrown or an arrow being shot. This orientation greatly aids in penetration of the particle/gene complex through the cell membrane and into the cytoplasm. In this study, the authors were able to achieve nearly 100% transfection levels in Bal17 B-lymphoma cells *in vitro*. Cell viability appeared to be unaffected up to 48 h post-transfection.

The use of magnetic nanoparticles to enhance the effectiveness of the cell-fusion vector hemagglutinating virus of Japan evenlope (HVJ-E) was demonstrated by Morishita and others. The group investigated protamine sulfate (PS)-coated magnetic nanoparticles and found that by associating these particles to HVJ-E, transfection was improved *in vitro* in BHK21 cells even with a reduction in the amount of HVJ-E and no evidence of toxicity.[8] However, direct injection of the complex into the livers of BALB/c mice showed that the PS-coated particles did not improve transfection levels, whereas the association of heparin-coated maghemite with the HVJ-E vector did.

Generally superparamagnetic iron oxide nanoparticles are used for the magnetic component of the complex. Superparamagnetic particles are strongly magnetic when placed in a magnetic field and hence are strongly attracted along the field gradient. In these iron oxides, the strong magnetization arises from the spin of unpaired electrons within the crystal lattice. These unpaired spins are coupled within the particle to produce an additive effect on the magnetic properties of the particles. However, their small size allows thermal energy to rapidly 'flip' the spins (this flipping frequency is on the order of 10^{-9}/s). Thus, when placed in a magnetic field, the magnetic energy of the system overcomes the randomizing thermal energy and the particles remain magnetized parallel to the field vector. However, when the field is removed, the particle's magnetization is lost.

The major advantage of using superparamagnetic particles is that in the absence of a field, they have less tendency to aggregate due to magnetic dipole interactions – particularly if the particles are in a core/shell configuration as this prohibits the magnetic cores from coming in close contact with one another. For *in vivo* uses, this is quite important as aggregation within the vasculature has the potential for problems such as embolization.

The recent refinement of magnetofection techniques demonstrates that it significantly reduces transfection time in comparison to other non-viral agents and has been used to successfully deliver small-interfering RNA and antisense oligonucleotides *in vitro* and *in vivo*

After the introduction of this technique by Mah and co-workers, Plank, Rosenecker and others further developed the technique and coined the term 'magnetofection'. A major achievement of this latter work was the demonstration of the potential of the technique for non-viral transfection. In the group's initial and subsequent studies, *in vitro t*ransfection time was reduced significantly in comparison to even lipid-based transfection agents while overall transfection levels were generally maintained.[9] The group has now successfully transfected a variety of both cell lines and primary human cells using magnetic nanoparticle-based transfection, including lung epithelial cells,[10] blood vessel endothelial cells,[11] keratinocytes, chondrocytes, osteoblasts, aminocytes,[12] and whole tissue samples of airways and blood vessels.[10,11]

Most recently, Schillinger *et al*.[12] have demonstrated the potential of magnetofection for delivering small-interfering RNA (siRNA). In this study, knockdown of luciferase reporter gene expression in the HeLa cell line was reported and expression was analyzed out to 72 h post transduction. The cell line had previously been stably transfected with luciferase using a retroviral vector. With interest in siRNA therapies increasing

rapidly, the use of magnetofection could prove to be an extremely useful mechanism for delivery.

Krötz and others have recently used magnetofection to successfully deliver antisense oligonucleotides both *in vitro* and *in vivo*. In their initial study,[13] antisense oligodesoxynucleotides (AS-ODN) were delivered to human umbilical vein endothelial cells (HUVEC) *in vitro* and *in vivo* via injection into the femoral arteries of male mice. Transfection levels of 84% were achieved in culture and fluorescence microscopy analysis of tissue samples from the mice indicated high levels of targeted transfection with minimal associated background fluorescence. The group then adopted the technique in order to deliver and study the effects of AS-ODN and siRNA on signalling pathways involved in superoxide generation by endothelial NAD(P)H-oxidase. In HUVEC, SH2-domain containing phosphatase-1 (SHP-1) was found to counteract NAD(P)H-oxidase activity, demonstrating the role of SHP-1 in endothelial antioxidative defense.[14]

Analysis of the transfection mechanism using endocytosis-blockers and transmission electron microscopy revealed that it is the same in magnetofection as in other systems – that is, clatherin-dependent endocytosis.[12,15] The group has also developed optimized PEI-coated magnetic nanoparticles that exhibit exceptional transfection results.

Oscillating magnet arrays show promise for further enhancing magnetic nanoparticle-mediated gene delivery

Recent work by our group has focused on the use of oscillating magnet arrays to enhance the overall efficiency of magnetofection. This technique would add energy to the transfection system by introducing an oscillating, lateral component of motion to the particles as they sediment onto the cell culture. Initial studies in HEK293T cells and H292 human lung epithelial cells indicate that the introduction of oscillating magnets increases transfection levels up to tenfold in comparison to static fields and produces overall levels of transfection that are significantly higher than those achieved with cationic lipid-based agents.

While the improved transfection ability of oscillating magnet arrays has been demonstrated in relatively few cell types, it is clear that the introduction of this extra motion to the particles enhances transduction when compared to static field magnetofection. In addition, the lateral motion of the particle/gene complex, which is primarily perpendicular to the translational force exerted on the particles by the field gradient, may prove useful for promoting penetration of the mucous lining in the lung and enable delivery of therapeutic genes for cystic fibrosis.[16]

Prospects

While magnetic targeting appears to hold significant potential for gene therapy, there are still major obstacles to employing this technique in the clinic. Perhaps, the problem that is most difficult to overcome is, as with magnetic targeting for drug delivery, that of scale-up. Studies conducted on small animals have shown great promise; however, in larger animals and humans, sites that are farther from the magnet source are more difficult to target. This is due to the fact that a high gradient is necessary to capture the particle/gene complex. This high gradient, however, also leads to a very rapid decay of field strength with distance from the magnet source. With current rare earth magnets, such as NdFeB magnets used in most studies, it is not feasible to capture particles at sites that lie more than a couple of centimetres deep.[2]

The possibility of inducing an embolism due to the aggregation of magnetic particles within the blood vessel is another potential problem. Magnetostatic interaction and the capturing of large numbers of particles in the field may lead to blockage of the blood vessel before the particle/gene complex can be extravasated. Work on overcoming these problems for both drug and gene delivery is continuing.

Conclusions

The efficacy of magnetic nanoparticle-based gene delivery has been demonstrated most clearly *in vitro*. As such, there is great potential for non-viral *in vitro* transfection of a variety of cell lines, primary cells and tissue explants using this method, and in fact, static-field magnetofection systems are already commercially available. The development of new particles and the optimization of magnetic field parameters is already beginning to show great promise for advancing this technique. In particular, the use of oscillating arrays of permanent magnets has been shown to significantly increase overall transfection levels even well beyond those achievable with cationic lipid agents. The use of carbon nanotubes also shows great promise; however, the potential for *in vivo* use may be more limited in the near-term due to the potential for toxicity. While scale-up to clinical application is likely to prove difficult for some targets, the potential for magnetofection to facilitate delivery of therapeutic genes *in vivo* remains enticing.

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