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Advances in Magnetofection – Magnetically Guided Nucleic Acid Delivery: a Review

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Abstract

During the last decade, nanomagnetic methods for delivering and targeting nucleic acids have been developed which are often referred to as magnetofection. Nucleic acids carry the building plans of living systems. As such, they can be exploited to make cells produce a desired protein, or to shut down the expression of endogenous genes or even to repair defective genes. Hence, nucleic acids are unique substances for research as well as therapy to exploit their potential, they need to be delivered into cells which can be a challenging task in many respects. Magnetofection provides a novel tool for high throughput gene screening in vitro and can help to overcome fundamental limitations to gene therapy *in vivo*. Magnetofection is nucleic acid delivery to cells, supported and site-specifically guided by the attractive forces of magnetic fields acting on nucleic acid shuttles (vectors) which are associated with magnetic nanoparticles. In a magnetofection procedure, self-assembling complexes of enhancers like cationic lipids with plasmid DNA and small interfering RNA (siRNA) are associated with magnetic nanoparticles and are then concentrated at the surface of cultured cells by applying a permanent inhomogeneous magnetic field.

Keywords: *Magnetofection, Magnetic Nanoparticles, Nucleic Acid Therapy, Magnetic Drug Targeting.*

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1 INTRODUCTION

Magnetofection is defined as the magnetically enhanced delivery of nucleic acids associated with magnetic nanoparticles. Magnetofection is a novel, simple and highly efficient method to transfect cells in culture. This method attempts to unite the advantages of the popular biochemical (cationic lipids or polymers) and physical (electroporation, gene gun) transfection methods in single system while excluding their inconveniences including low efficiency and toxicity (Boussif *et. al*., 1995;

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Cotton *et. al*., 1994). Magnetofection was invented by Christian Plank and Christian Bergemann and is registered as a trademark. It exploits magnetic force exerted upon gene vectors associated with magnetic particles to draw the vectors towards, possibly even into, the target cells. In this manner, the full vector dose applied gets concentrated on the cells within a few minutes so that 100% of the cells get in contact with a significant vector dose (Hughes *et. al*., 2001; Kanof *et. al*., 1999; Kasahara *et. al*., 1994).

1.1 Steps of Magnetofection Process

Magnetofection process used for enhanced delivery of biomolecules includes various steps such as:

- Biomolecules, such as nucleic acids including plasmid DNA and siRNA, are mixed with magnetic nanoparticles made of biodegradable iron oxides coated with cationic molecules in a simple one-step process to form a biomolecule / magnetic nanoparticle complex.
- The biomolecule / magnetic nanoparticle complex is added to the cells to be transfected in a standard multi-well plate.
- A magnetic force is applied beneath the cells to be transfected using a magnet array beneath the multi-well plate. This draws the biomolecule / magnetic nanoparticle complex onto cells on the bottom of the multi-well plate. The biomolecule is drawn towards, and delivered into, target cells, generally via endocytosis.
- This leads to rapid, efficient transfection without disturbing membrane architecture, causing chromosomal damage or leaving holes in cell membranes, therefore maintaining higher levels of cell viability. This is in stark contrast to physical transfection methods, including electroporation or biolistic methods, that can damage, create holes or electroshock the cell membranes causing cell death.
- The magnetic nanoparticles are biodegradable and non-toxic at the recommended doses.
- After delivery into the cells, the biomolecules are released into the cytoplasm by different mechanisms depending on the formulation used.

NanoTherics magnefect technology is unique in that the magnetic field created is designed to oscillate, which promotes more efficient uptake of the biomolecule into the cell and results in transfection efficiencies higher than other transfection techniques (Fouriki *et. al*., 2010; Hiemenz *et. al*., 2010; Finsinger *et. al*., 2000;)

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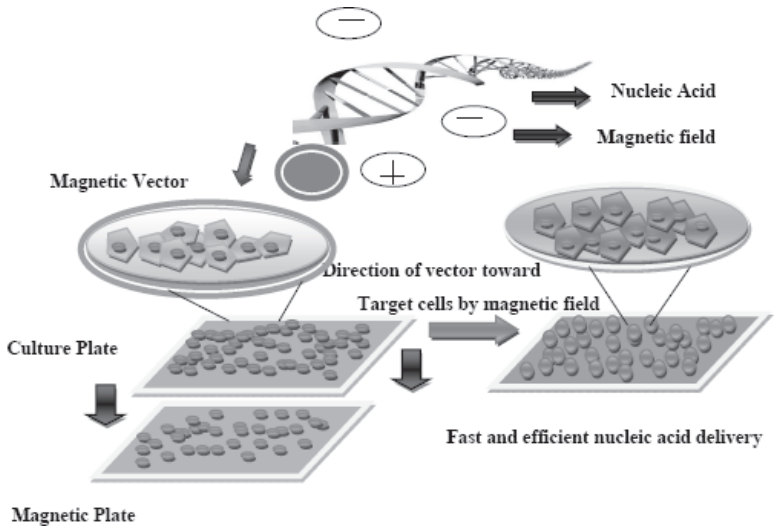

Figure 1: Principle of Magnetofection in Cell Culture (Schillinger *et. al*., 2005)

1.2 Principle of Magnetofection

The magnetofection principle is to associate nucleic acids with cationic magnetic nanoparticles. These molecular complexes are then concentrated and transported into cells supported by an appropriate magnetic field (Isner *et. al*., 1996; Kru¨ger *et. al*., 1994). Polyelectrolyte-coated magnetic nanoparticles are mixed with naked nucleic acids or synthetic or viral nucleic acid vectors in salt-containing buffer.

The particles associate with nucleic acids and vectors by electrostatic interaction and/or salt-induced colloid aggregation. The mixtures are added to cells in culture. The cell culture plate is positioned on a magnetic plate during 5–30 min of incubation. The magnetic field (s) rapidly sediment vectors on the cells to be transfected/transduced. In this way, the magnetic force allows a very rapid concentration of the entire applied vector dose onto cells, so that 100% of the cells get in contact with a significant vector dose.

1.3 Mechanism of Magnetofection

The magnetic nanoparticles are made of iron oxide, which is fully biodegradable, coated with specific cationic proprietary molecules varying upon the applications. Their association with the gene vectors (DNA, siRNA, virus, etc.) is achieved by salt-induced colloidal aggregation and electrostatic interaction. The magnetic particles are then concentrated on

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the target cells by the influence of an external magnetic field generated by magnets. The cellular uptake of the genetic material is accomplished by endocytosis and pinocytosis, two natural biological processes. Consequently, membrane architecture & structure stays intact, in contrast to other physical transfection methods that damage the cell membrane (Ma *et. al*., 2011; Fouriki *et. al*., 2010; Hiemenz *et. al*., 2010; Finsinger *et. al*., 2000).

The nucleic acids are then released into the cytoplasm by different mechanisms depending upon the formulation used:

1) Proton sponge effect caused by cationic polymers coated on the nanoparticles that promote endosome osmotic swelling, disruption of the endosom membrane and intracellular release of DNA form.

2) Destabilization of endosome by cationic lipids coated on the particles that release the nucleic acid into cells by flip-flop of cell negative lipids and charge neutralization.

3) Usual viral infection mechanism when virus is used. Magnetofection works for primary cells and hard to transfect cells that are not dividing or slowly dividing, meaning that the genetic materials can go to the cell nucleus without cell division. Coupling magnetic nanoparticles to gene vectors of any kind results in a dramatic increase of the uptake of these vectors and consequently high transfection efficiency.

4) Mechanism of oscillating nanomagnetic transfection

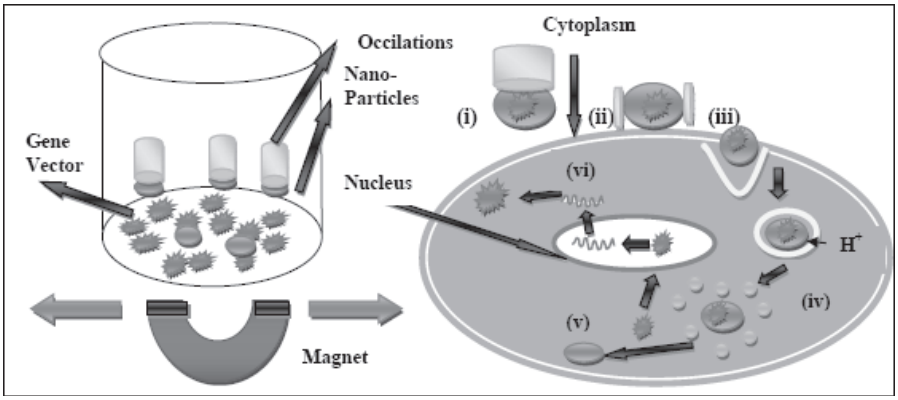

Figure 2: Proposed mechanism of oscillating nanomagnetic transfection (Fouriki *et. al*., 2010; Schillinger *et. al*., 2005)

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Plasmid DNA or siRNA is attached to magnet nanoparticles and incubated with cells in culture (left) (i)

An oscillating magnet array below the surface of the cell culture plate pulls the particle into contact with the cell membrane (ii) and drags the particles from side-to-side across the cells, (iii) mechanically stimulating endocytosis (iv) Once the particle/DNA complex is endocytosed, proton sponge effects rupture the endosome (v) releasing the DNA (vi) which then transcribes the target protein.

1.4 Technology: Magnetofection Reagents

(Li *et. al*., 1999; Leon *et. al*., 1998; Leventis *et. al*., 1990)

As the manufacturer of the magnetofection technology, chemicell offers two types of ready-to-use Magnetofection reagents.

PolyMAG is a universally applicable magnetic particle preparation for high efficiency nucleic acid delivery. It is mixed in a one-step procedure with the nucleic acid to be transfected and has been used successfully with plasmid DNA, antisense oligonucleotides and siRNAs.

CombiMAG is a magnetic particle preparation designed to be combined with any commercially available transfection reagent such as polycations and lipids and can be associated with plasmid DNA, antisense oligonucleotides, siRNAs or viruses. It allows you to create your own magnetic gene vector based on your favourite transfection reagent.

1.5 Advantages of Magnetofection

(Lu¨bbe *et. al*., 1998; Lu¨bbe *et. al*., 1996; Mendenhall *et. al*., 1996)

Greatly improved transfection rates in terms of percentage of cells transfected compared to standard transfection.

- Up to several thousand fold increased levels of transgene expression compared to standard transfections upon short-term incubation.
- High transfection rates and transgene expression levels are achievable with extremely low vector doses, which allow saving expensive transfection reagents.
- Extremely short process time. A few minutes of incubation of cells with gene vectors are sufficient to generate high transfection efficiency, compared to several hours with standard procedures.
- Magnet assisted transfection, or Magnetofection™, is a simple, highly effective method to transfect cells.

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- It is an easy-to-use, fast and efficient technology that promotes the uptake of biomolecules, such as nucleic acids including plasmid DNA and siRNA, into cells using magnetic nanoparticles and magnetic fields.
- In cell culture, magnetic vectors are magnetically sedimented on the target cells within minutes. Thus, the diffusion barrier to nucleic acid delivery is overcome, the full vector dose comes in contact with the target cells, and introduction of genetic material is synchronized.
- Nucleic acid delivery is greatly accelerated and its efficiency with many, if not most, vector types is improved. Magnetofection is applicable to small and large nucleic acids.
- Low-dose requirements, the possibility of confining nucleic acid introduction to a localized area (magnetic targeting), and the amenability to high-throughput automation. Due to the favorable dose-response profile and the rapid kinetics, vector-related toxicity can be kept low.
- Magnetic nanoparticles for delivery of nucleic acid to target cells can use either non-viral or viral vectors.

1.6 Applications of Magnetofection

This has several important consequences:

- Magnetofection can be utilized to deliver synthetic siRNAs to cultured cells. Certain magnetic nanomaterials associate with siRNAs and are suitable for siRNA delivery, either alone or in combination with cationic polymers or cationic lipid enhancers; these complexes are targeted to the

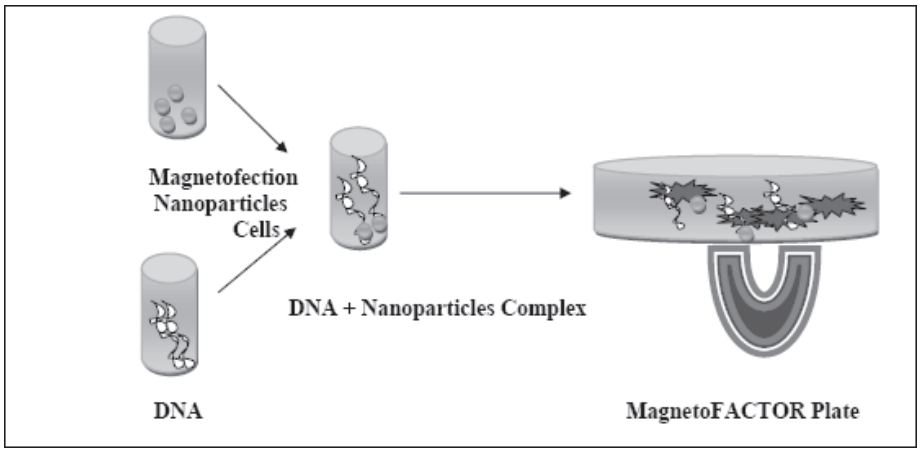

Figure 3: Magnetic nanomaterials associated with DNA (http://www.bocascientific.com)

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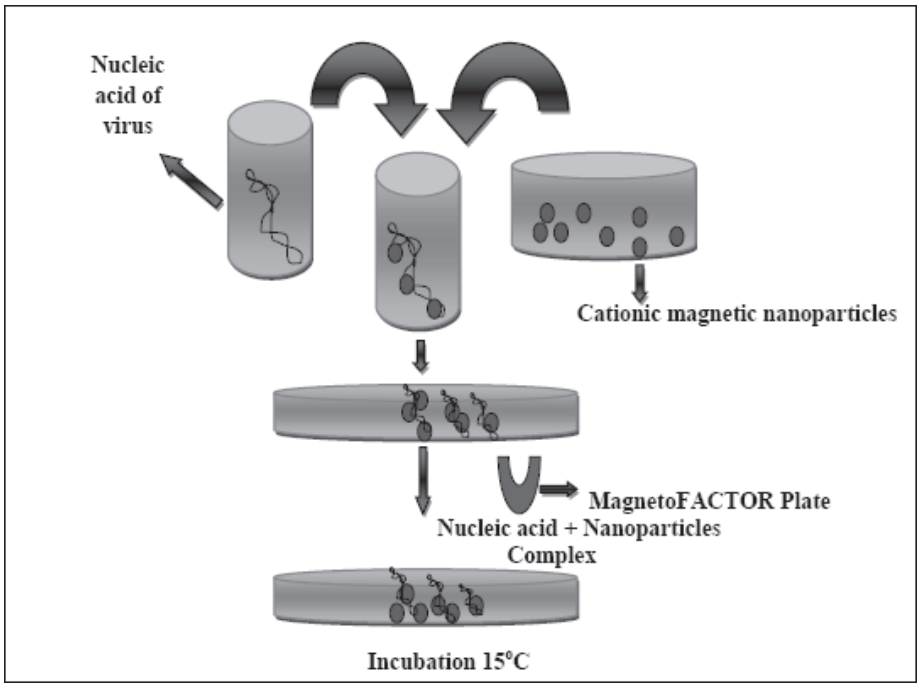

Figure 4: Magnetofection for Virtal DNA transfection (www.chemicell.com; www.ozbiosciences.com)

cell surface by application of a gradient magnetic field (Campbell *et. al*., 2012; Mair *et. al*., 2009; Luo *et. al*., 2000)

- Magnetofection is used for nucleic acid delivery by magnetic force acting on magnetic particles and nucleic acids or nucleic acid vectors. Vectors are bound to magnetic, usually iron oxide, nanoparticles, in most cases by non-covalent bonds. Magnetic force accumulates and/or holds magnetic vectors in a target tissue against hydrodynamic forces (Mair *et. al*., 2009; Luo *et. al*., 2000; Widder *et. al*., 1983).
- Magnetofection has been adapted to all types of nucleic acids (DNA, siRNA, dsRNA, shRNA, mRNA, ODN), non-viral transfection systems (transfection reagents) and viruses. It has been successfully tested on a broad range of cell lines, hard-to-transfect and primary cells (Mykhaylyk *et. al*., 2008; Tomko *et. al*., 1997; Plank *et. al*., 1994).
- Ocean NanoTech has developed superparamagnetic nanocrystals made of iron oxide that are 5 nm to 50 nm in diameter that are ideal for magnetofection. The surfaces of the superparamagnetic nanocrystals are modified with biocompatible polymers with specific proprietary

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anionic and cationic molecules that vary upon the applications. The superparamagnetic nanocrystals carrying the gene vectors are used to transfect the target cells through the influence of an external magnetic field. The cellular uptake of the genetic material is accomplished by two natural biological processes, endocytosis and pinocytosis, induced by the applied magnetic field. Use of the 5 or 10 nm superparamagnetic nanocrystals from Ocean NanoTech allows the membrane architecture & structure to remain intact unlike other bigger nanoparticles and physical transfection methods that damage the cell membrane. Small DNA sequences and plasmid are being introduced to the iron oxide nanocrystals which will subsequently be used to transfect specific cells (Pickles *et. al*., 2000; Meyer *et. al*., 1995; Wu *et. al*., 1987).

- New magnetic technologies have been developed to improve the uptake and expression of DNA and siRNA in cells growing in culture (Sukoyan *et. al*., 2013; Plank *et. al*., 2003; Plank *et. al*., 1999).
- Gene vectors were associated with super-paramagnetic nanoparticles and targeted gene delivery by application of a magnetic field. This potentiated the efficacy of any vector up to several hundred-fold, allowed reduction of the duration of gene delivery to minutes, extended the host tropism of adenoviral vectors to non-permissive cells and compensated for low retroviral titer (Scherer *et. al*., 2002; Povey *et. al*., 1986).

1.7 Biodistribution of magnetic nanoparticles

- The biodegradable cationic magnetic nanoparticles are not-toxic at the recommended doses and even at higher doses. Gene vectors / magnetic nanoparticles complexes are seen into cells after 10–15 minutes that is much faster than any other transfection method. After 24, 48 or 72 hours, most of the particles are localized in the cytoplasm, in vacuoles (membranes surrounded structure into cells) and occasionally in the nucleus (Plank *et. al*., 2011; Scherer *et. al*., 2002; Shayakhmetov *et. al*., 2000; Povey *et. al*., 1986).

2 CONCLUSION: FUTURE PERSPECTIVES OF MAGNETOFECTION

A very exciting future perspectives of magnetofection is to use it therapeutically e.g. in tumor targeting or local neo-vessel formation. Apart from direct injection into the target tissue, the injection into blood vessels which are rather distant to the target site is assumed to become the most important form of vector administration for therapeutic magnetofection.

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Iron oxide nanoparticles can be used as "superparamagnetic", which means that they are strongly attracted to a magnetic field but they do not retain residual magnetism after the field is removed. Therefore they cannot agglomerate (like ferromagnetic particles) after removal of the magnetic field. It has been found that iron oxide particles used as contrast agents in magnetic resonance imaging (MRI) are fully biocompatible.

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