MAGNETOFECTION™, The tool of choice to reach high transfection and transduction efficiency with low toxicity on Primary cells

INTRODUCTION

Magnetofection™ was developed to gather in one convenient system the advantages of the popular biochemical (cationic lipids or polymers) and physical transfection methods (electroporation, gene gun) while overcoming their respective limitations such as low efficiency, toxicity, difficulty to handle .... Magnetofection can be defined as the delivery of nucleic acids, either 'naked' or packaged (in complexes with lipids or polymers, and viruses), using magnetic nanoparticles (MNP) under the guidance of an external magnetic field [1] [2] [3].

HOW DOES IT WORK?

The MNP are made of iron oxides, which are fully biodegradable, coated with specific proprietary cationic molecules varying upon applications. These MNP are complexed with nucleic acids, transfection reagents or viruses depending on the applications. The magnetic complexes are then concentrated onto cells by the influence of an external magnetic field generated by a specific magnetic plate. The cellular uptake of the genetic material is accomplished by endocytosis and pinocytosis, two natural biological processes [4]. Consequently, membrane architecture and structure stay intact. The magnetic force exerted upon the gene vectors allows a very rapid concentration of the entire applied vector dose onto cells/organs, so that 100% of the target cells get in contact with a significant vector dose. [2][3]

The protocol is a very straightforward and easy procedure (Fig. 1):

1. Solutions of nucleic acids, viral particles or lipoplexes/polyplexes are prepared in buffer or serum-free culture medium
2. Vectors are mixed with magnetic nanoparticles formulation composing the Magnetofection™ reagents and incubated 20-30 minutes at room temperature (RT).
3. Magnetic complexes are added directly onto cells. Then, the cell culture dish is placed on a magnetic plate for 5 to 20 minutes
4. Magnetic plate is removed and cells are cultured until experimental assay.

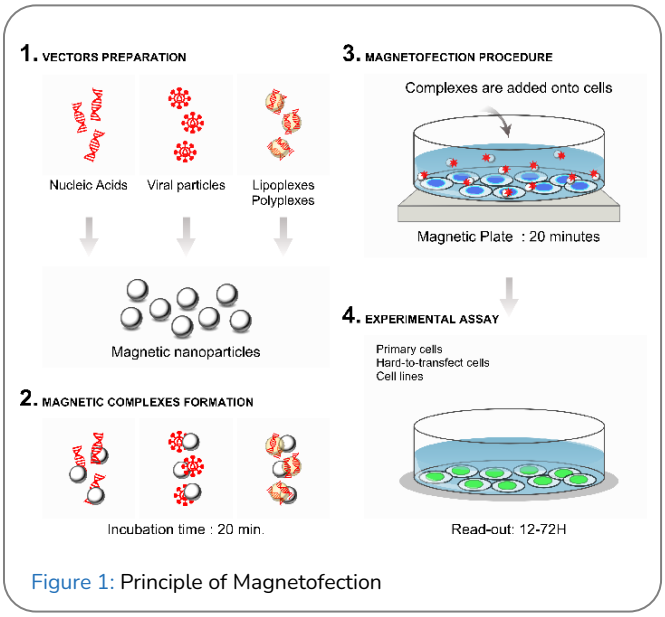

Figure 1: Principle of Magnetofection

BIODISTRIBUTION OF MAGNETIC NANOPARTICLES

The MNP are not toxic at the recommended doses and even higher. Toxicity of these iron-based particles has been extensively studied [5] mainly due to their widespread scientific, diagnosis and medical use (cell separation, MRI, hyperthermia or cancer therapies...). *In vitro*, gene vectors / MNP complexes are internalized into cells after 10-15 minutes i.e. much faster than any other transfection methods. After 24, 48 or 72 hours, most of the particles are localized in the cytoplasm, in vacuoles and occasionally in the nucleus. In addition, MNP do not influence cell function. *In vivo*, without the application of an external magnetic field, MNP accumulates preferentially into the spleen, lung and liver (although distribution depends on the charge, size and composition of the particles), and degradation occurs through natural iron metabolism pathways.

WHAT ARE THE APPLICATIONS?

Magnetofection™ is the only versatile and universal technology to transfect or transduce cells, adapted to *in vitro* or *in vivo* applications and all types of nucleic acids (DNA, siRNA, dsRNA, shRNA, mRNA, ODN...) as well as viruses.

Tailored reagents are available including the popular NeuroMag, specific for neurons transfection, and the whole range of ViroMag reagents, to enhance transduction or infection.

NON VIRAL APPLICATIONS	
PolyMag | PolyMag Neo	Polymer complex for all nucleic acids transfection
CombiMag	Improves the efficiency of any transfection reagents
Magnetofectamine O2 Kit	For all nucleic acids - Association of CombiMag + MTX transfection reagent
NeuroMag	For Neurons transfection
Glial-Mag	For Glial cells transfection
SilenceMag	For siRNA applications
FluoMag	Fluorescent Magnetofection reagents
VIRAL APPLICATIONS	
ViroMag	For enhancing viral transduction efficiency (suitable for all viruses)
ViroMag R/L	For Lentiviral and Retroviral transduction
AdenoMag	For Adenoviral and AAV transduction
Mag4C-LV / Mag4C-AD	For capturing and concentrating Lentiviruses and Adenoviruses
***In vivo* APPLICATIONS**	
In vivo PolyMag & DogtorMag	For all nucleic acids
In vivo ViroMag	For enhancing viral transduction efficiency
In vivo SilenceMag	Specifically designed for *in vivo* siRNA transfection

PRIMARY CELLS SUCCESSFULLY TRANSFECTED USING MAGNETOFECTION

Magnetofection™ has been successfully tested on a broad range of cell lines, hard-to-transfect and primary cells (Fig. 2) [2][6][7]. It is perfect for non-dividing or slowly dividing cells, meaning that the genetic materials can go to the nucleus without cell division. It has been shown that combining magnetic nanoparticles to gene vectors of any kind results in a dramatic increase of uptake of these vectors and high transfection efficiency.

It is the only technology suitable both for viruses and non-viral nucleic acid delivery applications:

- For non-viral nucleic acid delivery, it is ideal for primary and hard-to-transfect adherent cells.
- For viral applications, it is ideal for any cells including primary cells (adherent and suspension).

Using Magnetofection, up to 75 % transfection efficiency can be achieved in many primary cells, such as Chondrocytes, Endothelial cells (HUVEC, HMEC), Epithelial cells, and Fibroblasts.

NeuroMag reagent is now routinely used in numerous laboratories to transfect any kind of primary neurons (hippocampal, cortical, motor neurons, dorsal root ganglion, neural stem cells.....)[8][9]. Magnetofection is also highly efficient on classic and hard-to-transfect cell lines, such as SH-SY5Y, PC-12, MEF, C6, etc...

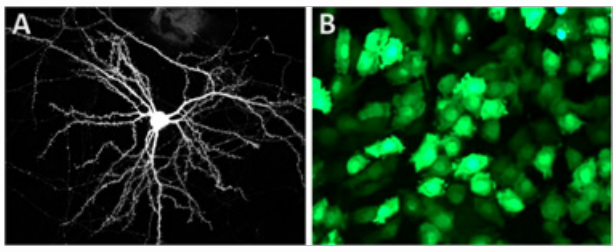

Figure 2: Example of primary cells transfected by Magnetofection. (A) primary mouse hippocampal neurons (DIV 21) were efficiently transfected using NeuroMag™. Photo was kindly provided by C. Charrier [9]. (B) Primary HUVECs were transfected using pVectOZ-GFP and PolyMag Neo™.

MAGNETOFECTION MINIMIZES TOXICITY

Magnetofection™ is effective even with low doses of nucleic acids resulting in minimized cytotoxicity (Fig. 3). As an example, a combination of two technologies, Lipofectamine™ 2000* and CombiMag (Magnetofection™ reagent), enables using smaller amounts of nucleic acids and reagent while increasing the overall efficiency of your transfection.

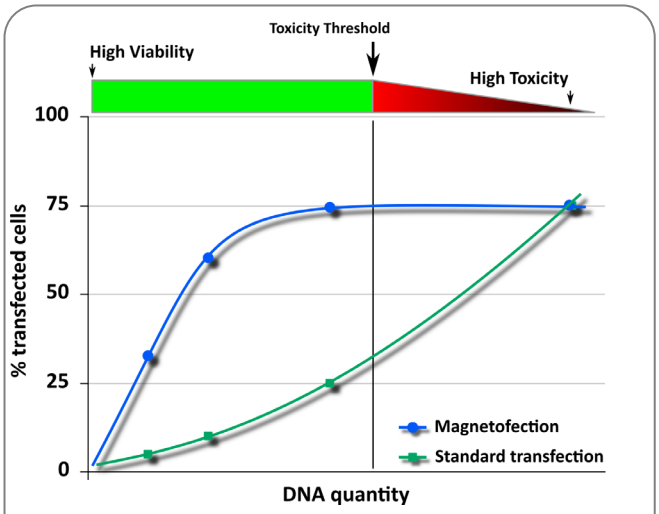

Figure 3: Viability and Efficiency – Magnetofection using CombiMag reagent, minimized toxicity compared to Lipofectamine™ 2000* alone. High transfection efficiency is achieved with less nucleic acid amount.

Magnetofection™ has also been compared to electroporation. Electroporation is one of the most efficient transfection tool but also known to generate high cytotoxicity. The mechanism of electroporation is the creation of nanometer-scale water-filled holes in the membrane that cause toxicity. It has been showed that Magnetofection can be as efficient as electroporation but cytotoxicity will be significantly lower (Fig. 4).

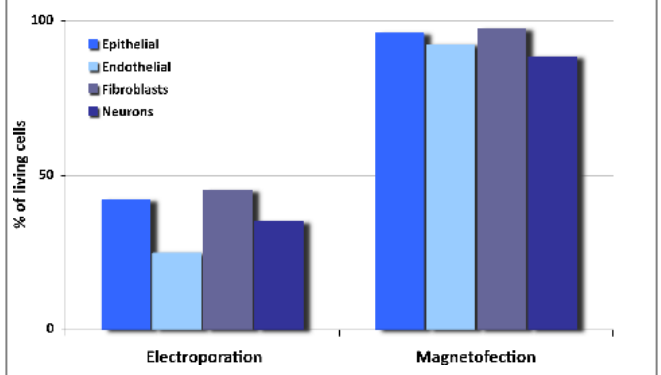

Figure 4: Cytotoxicity comparison on primary cells between 2 transfection methods: Electroporation and Magnetofection.

MAGNETOFECTION IS ALSO POWERFUL FOR *IN VIVO* APPLICATIONS

Magnetofection™ has been tested on several animal models and different organs (such as brain, lung, stomach) and tumors such as fibrosarcoma [2]. Researchers showed that injection of magnetofection complexes into mice tumors enables to slow down a tumor growth by 50%. "*^{131}I-hVEGF siRNA/SilenceMag exhibited an antitumor effect. The synergic therapy of ^{131}I-hVEGF siRNA/SilenceMag might be a promising future treatment option against HCC with the dual functional properties of tumor therapy and imaging*".[10]

As for another example, researchers have transfected DNA plasmid in rat visual cortex neurons using NeuroMag, a Magnetofection reagent dedicated to neural cells. The transfection rates reached values of up to 97% after 30 days, comparable to those achieved by viral vectors.[11]

MAGNETOFECTION INCREASES TRANSDUCTION EFFICIENCY

Magnetofection™ is also a successful method in enhancing viral transduction efficiency. ViroMag, ViroMag R/L and AdenoMag, the 3 magnetofection reagents dedicated to viruses, enable to increase transduction efficiency up to 10 fold compared to virus alone (Fig. 5). In addition, this technology accelerates and synchronizes the transduction process, and enables to concentrate the viral dose onto cells for optimal performance.[12] [13][14].

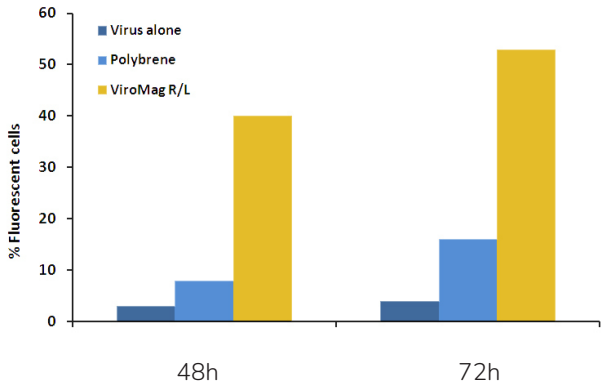

Figure 5: ViroMag R/L is highly efficient for lentiviral infection. NIH-3T3 were infected with a lentivirus coding for GFP alone or with Polybrene and ViroMag R/L. Percentage of infected cells was determined 48 and 72H after infection by FACS analysis.

COMBIMAG MAGNETOFECTION BEADS INCREASE THE EFFICIENCY OF ANY TRANSFECTION REAGENTS

CombiMag magnetic nanoparticles have been designed to be associated with any commercial transfection reagent and can be used with all types of nucleic acids. It allows creating your own optimal delivery system with 30% to 500% transfection efficiency enhancement. It has been tested with several transfection reagents such as Lipofectamine 2000* and Fugene 6** (Fig. 6).

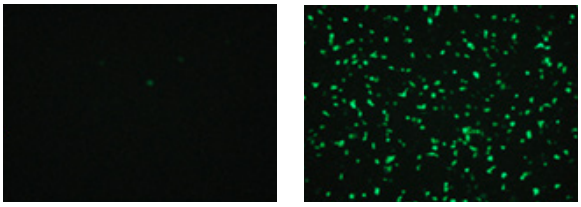

Figure 6: Effect of CombiMag on the Fugene 6 transfection reagent. The addition of CombiMag with Fugene 6 showed a high transfection efficiency increase on primary human keratinocytes.

Recently, researchers showed that magnetofection in combination with a lipid reagent is a very efficient transfection methods in MEF cells.

«*In our analysis, Magnetofection (CombiMag), together with lipid reagents, resulted in a 3- to 13-fold increase in transfection efficiency compared with the lipid reagent alone.*»[15]

AN AFFORDABLE METHOD FOR ANY LABORATORY

The only requirement for Magnetofection™ is a magnetic plate specifically designed for this application. The magnetic plate is a one-time buy and completely reusable. There is no need for expensive equipment contrary to approaches such as electroporation or gene gun.

* Lipofectamine® is a registered trademark of Life Technologies, Inc

** Fugene is a registered trademark of Fugent LLC.

CONCLUSION

The different studies carried out demonstrate that Magnetofection™ is a powerful method for introducing nucleic acids into cells. Indeed, magnetic targeting was successful in overcoming the limitations encountered by traditional drug delivery systems: a low concentration of vectors on target cells, a potentially high toxicity of synthetic vectors and an uncontrolled immune response and complement activation following systemic diffusion of viral carriers *in vivo*.

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