In vivo Magnetofection™ – Results

The main problems currently associated with systemic gene vector administration (gene therapy) include biodistribution of gene vector throughout the body, lack of specificity towards a pathological site (bioavailability at the target site), necessity of a large dose to achieve high local concentration, non-specific toxicity, inactivation of vectors due to undesired interactions with components of the *in vivo* milieu (opsonisation, interaction with complement or immune systems etc.) and local other adverse side effects due to high vector doses.

Magnetofection™ is defined as nucleic acid delivery under the influence of a magnetic field acting on nucleic acid vectors that are associated with magnetic nanoparticles. This technology has been developed mainly to solve the problem related to diffusion limited process for *in vitro* applications and to restricted bioavailability at the target site for *in vivo* applications.

In vivo Magnetofection™ has been designed for *in vivo* targeted **transfection** of various types of nucleic acids such as DNA, RNA, oligonucleotides (*In vivo* PolyMag / *In vivo* DogtorMag) or **transduction** with any viral vectors (*In vivo* ViroMag). DNA/ or virus/nanoparticles complexes can be easily administrated through various injection routes such as systemic administration (intravenous, intra-artery) or local administration (intratumoral, intracerebroventricular).

In vivo Magnetofection™ main advantages in comparison to other procedures:

1. Increased transfection/transduction efficiency
2. Targeted process (magnetically-driven)
3. Reduction of the systemic dissemination of vectors during injection
4. Reduction of the vector doses (nucleic acid, virus...)
5. Work under non permissive conditions (hypothermia, physiological flow conditions)
6. Penetration of the vector into tissues
7. Minimized toxicity
8. Universal – suitable for all nucleic acids or viral vectors

Sucessful in vivo applications

Tissue	Animal	Vector	Suitable Reagent	Type of Administration	References
Non viral applications					
Ear artery	Rabbit	DNA	*In vivo* PolyMag	intra-artery	1
Ear artery	Pig	DNA	*In vivo* PolyMag	intra-artery	1
Muscle blood vessels	Mouse	ODN	*In vivo* PolyMag	intra-artery	2
Myocard	Pig	DNA	*In vivo* PolyMag	intra-artery	Not published
Myocard/endothelial cells	Mouse	DNA	*In vivo* PolyMag	i.v.	10
Breast tumor	Hamster	DNA	*In vivo* PolyMag	intra-tumoral	4
Fibrosarcoma	Cat	DNA	*In vivo* PolyMag	intra-tumoral	4,5,6,7
Subcutaneous tumor	Mouse	siRNA	*In vivo* SilenceMag	intra-tumoral	8
Subcutaneous tumor	Mouse	DNA	*In vivo* Dogtor	intra-tumoral	9
Intestine	Rat	DNA	*In vivo* PolyMag	intra-lumen	3
Urogenital ridges	Mouse	DNA	*In vivo* Dogtor	*ex-vivo*	10
Lung	Mouse	DNA	*In vivo* PolyMag	aerosol	13
Skin	Mouse	DNA	*In vivo* PolyMag	s.c.	12

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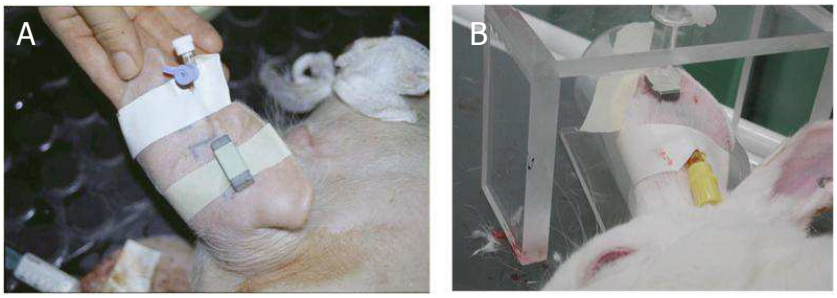
Tissue	Animal	Vector	Suitable Reagent	Type of Administration	References
Viral applications					
Embryo brain	Rat	Retrovirus	*In vivo* ViroMag	i.c.v.	14
Embryo brain	Rat	Lentivirus	*In vivo* ViroMag	i.c.v.	15
Stomach	Mouse	Adenovirus	*In vivo* ViroMag	intra-lumen	3
Heart	Rat	Lentivirus	*In vivo* ViroMag	*ex-vivo*	16
Endothelial cells	Mouse	Lentivirus	*In vivo* ViroMag	*ex-vivo*	17

In vivo Magnetofection™ – Non Viral Applications

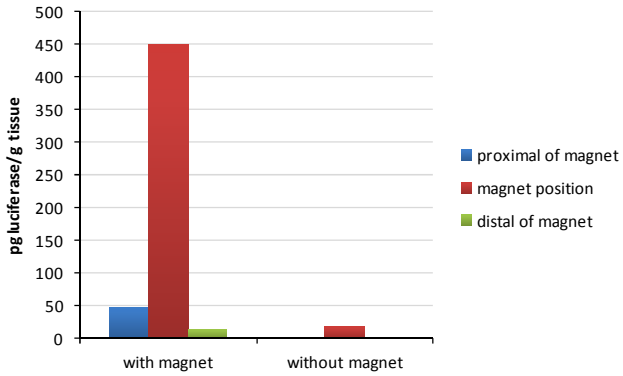
Tissue targeting

In vivo PolyMag – gene delivery

Transfection of ear artery endothelial cells. In this study, Magnetofection™ has been used for local gene delivery in blood vessels. Plasmid DNA encoding luciferase complexed with **PolyMag** magnetic nanoparticles were injected into the ear veins of pig and ear artery of rabbit A permanent magnet was placed respectively upstream and downstream the blood flow circulation and near the injection sites as shown in the photo hereunder. Results demonstrated that transgene was expressed at the magnet position indicating an efficient magnetic targeting.



Authors injected into the ear veins of pig (A) or the ear artery of rabbit (B) a plasmid DNA encoding luciferase mixed with *in vivo* **PolyMag**. The magnet was positioned onto the circulation route from the injection site during the injection and the following 60 min.

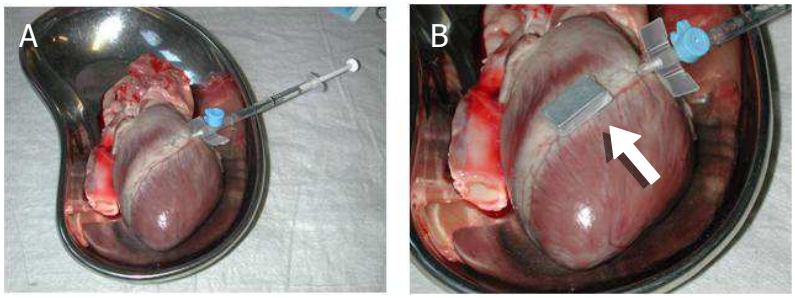


After 42h, reporter gene expression was found primarily at the magnet position site and to a lesser extent proximal and distal of the magnet. As control, the same vector composition was injected in the contralateral vessel without application of a magnet. No significant reporter gene expression was found at the topographically analogous positions. *From Plank et al., Expert Opin Biol Ther., 2003; 3:745-58 (1)*

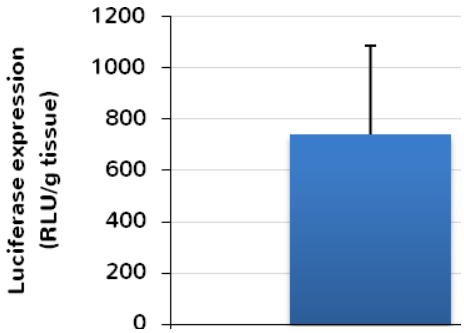
The results show the efficiency of *In vivo* PolyMag for DNA delivery to a targeted area

Transfection in heart or cardiovascular systems. Gene therapy in cardiovascular system faces some inconveniences such as direct myocardial injection that may harm the cardiac tissue. In addition, the strong hydrodynamic forces upon the vector/DNA complexes exerted by the circulating blood lead to low-transfection efficiency following intracoronary injection.

Non viral nucleic acids delivery in the cardio-vascular system has been demonstrated in previous studies (1, 2, 3).

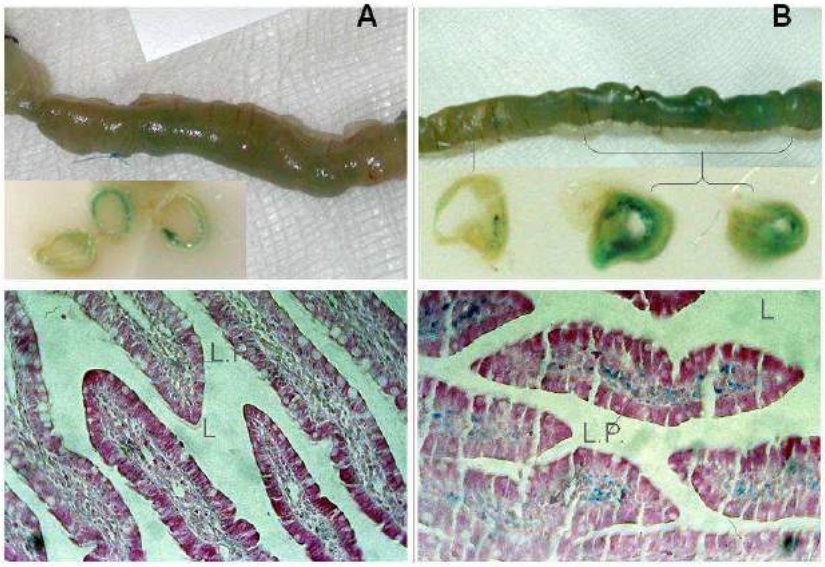


In this ex-vivo study, pig hearts was infused through coronary artery with complexes of DNA encoding for luciferase and **PolyMag** (A). A permanent magnet was applied on the myocardial surface for 20 minutes (B). 48h later, explants of myocardial tissue were prepared and luciferase expression was analysed.



Variable luciferase expression (741.9 ± 346.5 pg luciferase/g tissue) was found in vessels under direct influence of magnetic field.

Transfection in rat intestine. Gene delivery and gene therapy in the guts are particularly challenging due to stringent conditions (abundance of degradative enzymes, presence of degraded nutriments and bacteria...). In their study, Scherer and coll. successfully transfected a plasmid DNA encoding Lac-Z gene complexed with **PolyMag** magnetic nanoparticles into guts. Complexes were injected into the ileum lumen of rat and a permanent magnet was applied for 20 minutes.

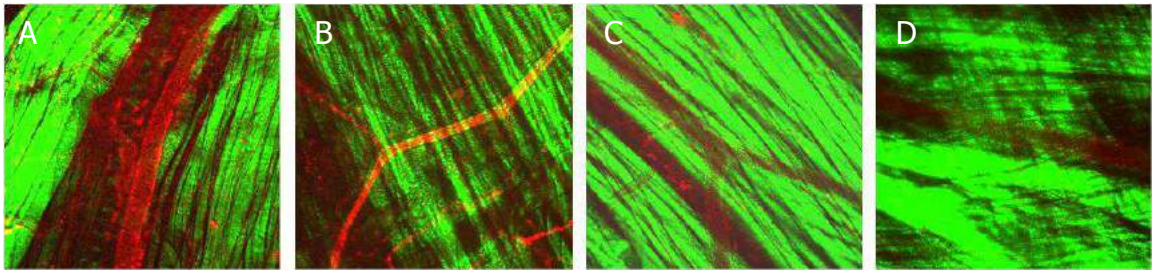


Complexes of DNA and *in vivo* **PolyMag** nanoparticles were injected into the ilea of rats in absence (A) or under the influence of a magnetic field (B). X-Gal staining was performed after 48h and revealed an highly efficient gene delivery in the presence of magnet (B) at the macroscopic level (upper panel) and at the microscopic level (lower panel). In absence of magnetic field, only rare transfection events were observed (A). *From Scherer et al., Gene Therapy., 2002; 9:102-109 (3)*

The results show the efficiency of *In vivo* PolyMag for DNA delivery in hard-to-transfect tissues within highly stringent conditions

In vivo PolyMag – oligonucleotides delivery

Transfection of cremaster muscle vessels. Mice were infused with Cy3-labeled antisense ODN complexed to **PolyMag** through a femoral catheter and a permanent magnet was positioned on the cremaster muscle. Results showed that antisense ODN delivery was efficiently directed to specific vascular site corresponding to the magnet location.

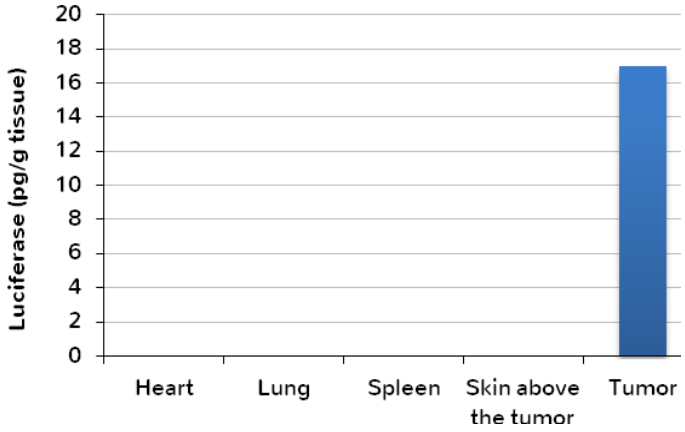


To investigate a strategy for directing antisense ODN to one specific vascular site after intra-arterial injection, Krötz and coll. infused mice with Cy3-labeled antisense ODN complexed to **PolyMag** through a femoral catheter. During and for 4 min following infusion, only the right cremaster muscles were exposed to a magnetic field. At 24 h, all large (A) and smaller arterioles (B) showed high levels of fluorescence only in right cremaster muscles. This was not the case in cremaster vessels of the contralateral testis in identical animals (C and D). In control vessels, only a few large arterioles, and none of the small arterioles, showed fluorescence. The results indicated specific targeting and uptake of fluorescently labelled antisense-ODN into the vessels induced by Magnetofection. *From Krötz et al., Mol. Ther., 2003; 7:700-710 (2).*

The results show the efficiency of *In vivo* PolyMag for ODN delivery

In vivo PolyMag – gene delivery

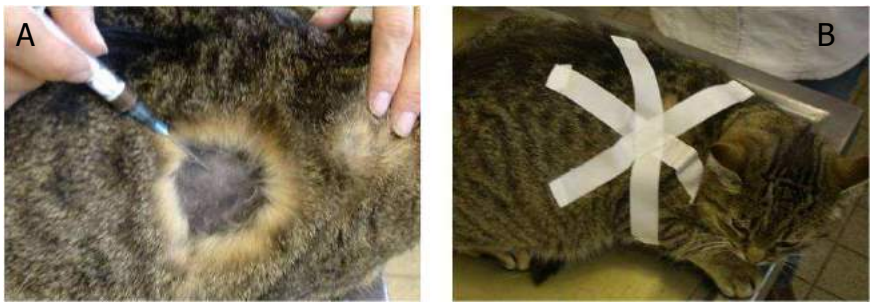
Transfection of tumor in hamster. Schillinger and coll. have injected directly into the tumor, a plasmid DNA encoding luciferase complexed to **PolyMag** and positioned a permanent magnet onto the tumor during for 1 hour. Results showed that only tumoral tissues expressed reporter gene indicating that magnetic guidance is able to hold the injected dose within the tumor in order to enhance intratumoral gene delivery.



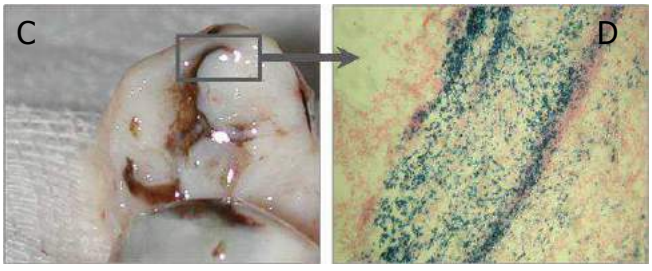
Spontaneous hamster breast tumors were injected with plasmid encoding luciferase complexed with **PolyMag** magnetic nanoparticles. A magnet was fixed on the tumor adjacent to the site of injection during one hour. 40 h later, expression of luciferase was measured only in the tumor and not in any of the other organs examined. *From Schillinger et al., J. Magn. Magn. Mater.,2005; 293:501-508 (4)*

The data show that local gene delivery can be achieved in tumoral tissue by *in vivo* Magnetofection

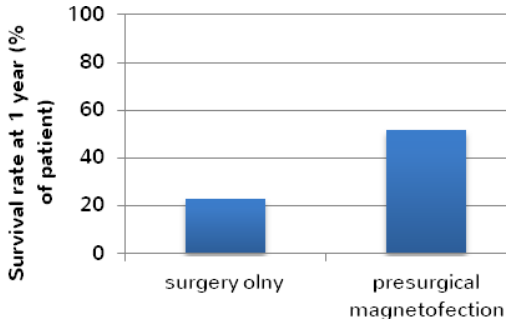
Transfection of tumor in cat. Magnetofection™ has been successfully used in veterinary clinical studies for the treatment of feline fibrosarcoma using immunostimulatory gene therapy. Feline fibrosarcoma is one of the most common feline tumors with a relapse rate of 75% within 6 months upon surgical resection. In phase I and phase II trials, gene therapy was conducted on cats receiving pre-operative intratumoral injection of plasmid encoding granulocyte-macrophage colony-stimulating factor and magnetic nanoparticles. These studies showed no adverse events as well as an increase in animal survival (5, 6, 7).



After intratumoral injection of the plasmid-attached nanoparticles (A), a magnetic field was applied directly onto the tumor for magnetic guidance of magnetic beads-associated plasmid (B).



Tumor biopsy (C) revealed a restricted targeting of the magnetic nanoparticles stained in blue. In (D), a magnification of the square zone in (C) confirms the efficiency of the delivering by cellular staining. *From Schillinger et al., J. Magn. Magn. Mater.,2005; 293:501-508 (4)*

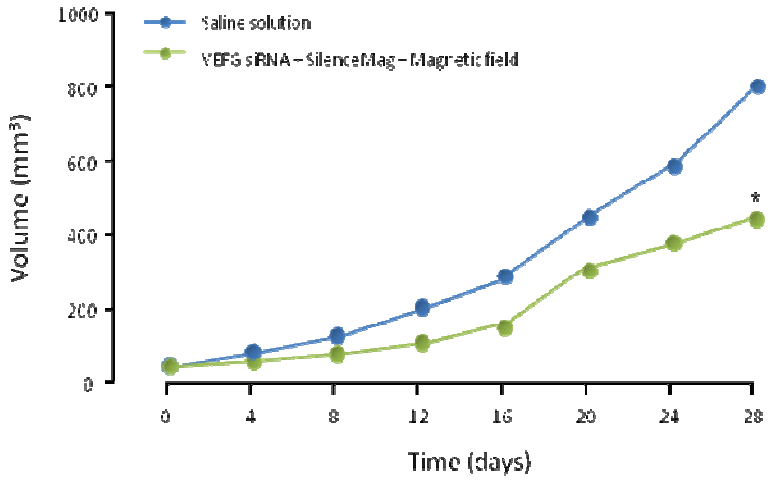


The preliminary clinical outcome of the phase II study demonstrated a significant increase in tumor-free survival of the cats from only 23% at the 1 year time point in the case of standard therapy (surgery only) to 52% with pre-surgical Magnetofection of the human GM-CSF. *From Schillinger et al., Mol. Ther 2006;. 13:105 (5)*

Intratumoral Magnetofection is efficient for gene delivery in veterinary clinical studies

In vivo SilenceMag – gene silencing

Transfection of subcutaneous tumor in mouse. Chen and her collaborators have shown that the use of ***in vivo*** **SilenceMag** coupled to the application of an external magnetic field increases the attraction and the retention of siRNA directed against vascular endothelium growth factor (VEGF) at the site of the tumor. Biodistribution and cytotoxicity of siRNA/SilenceMag was assessed by SPECT and MRI and tumor size was evaluated daily (8).



Subcutaneous tumors were generated by injection of hepatocarcinoma tumor cells into the right flank of immunosuppressed mice. Tumor growth was then monitored daily after intravenous injection of VEGF/SilenceMag. *From Chen et al, BMC Cancer, 2014; 14:114 (8)*

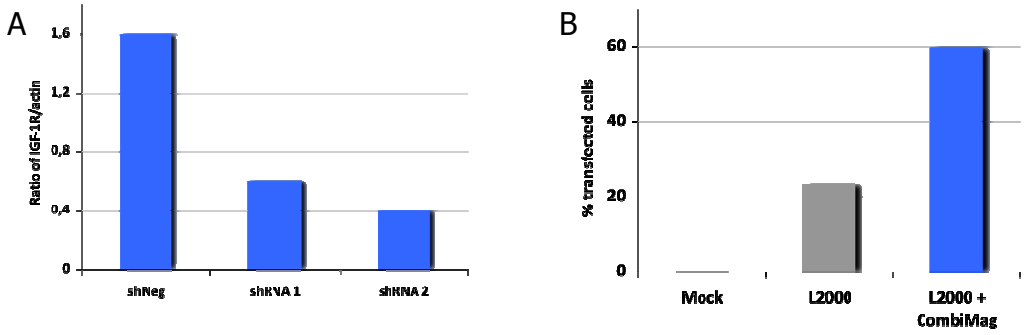
Magnetically assisted delivery of siRNA is a tool of choice for tumor therapy studies

In vivo DogtorMag-like – gene silencing

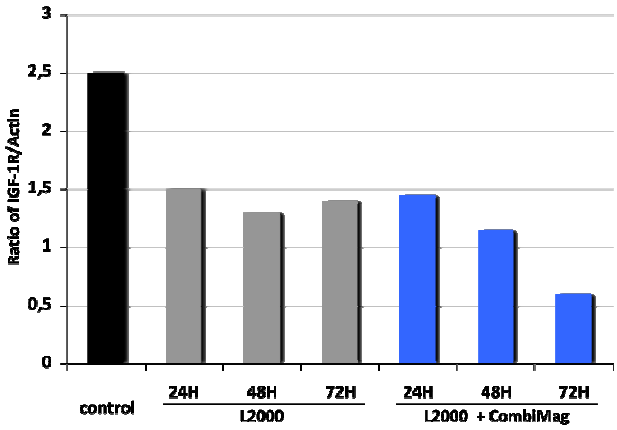
Ex vivo transfection of urogenital ridges. Svingen and coll. established a new protocol for the study of gene function during mouse development without the generation of knock-out animal models. The authors performed Magnetofection™ on developing genital ridges from mouse embryo between 11.0 and 13.0 dpc. Magnetofectamine kit was used : explants were injected with 1 µg DNA/2.5 µL Lipofectamine™ 2000/3.5 µL

CombiMag, placed for one hour on magnetic plates and then cultured for 36-72h on agar blocks. In these conditions, gene expression was observed after 4h and still detectable after 5 days in culture. (9)

Transfection of tumor in mice. Recently, Wang and coll. successfully associated CombiMag and Lipofectamine™ 2000 (Magnetofectamine kit) to transfect targeted areas *in vivo*.



First, the authors confirmed the efficiency of the Magnetofectamine™ kit *in vitro*: (A) IGF-1R gene expression in human A549 cells after transfection using Magnetofectamine kit with two plasmids encoding for shRNA directed against gene of interest. (B) Lipofectamine alone (L2000) and Magnetofectamine were used with a GFP encoding plasmid in A549 cells.



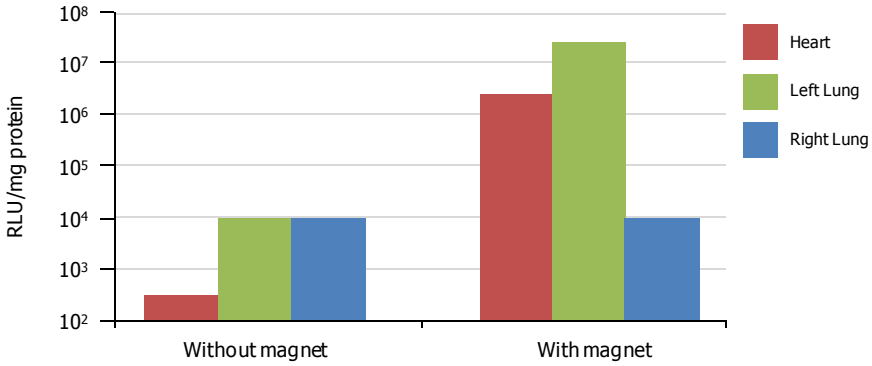
Then the authors silenced IGF-1R protein expression into A549-induced tumors *in vivo*. After tumour induction by A549 cells subcutaneous injection, PBS (control), 50µg plasmid encoding shRNA/125 µL Lipofectamine™ 2000 (L2000) and 50 µL shRNA encoding plasmid/50 µg CombiMag/125 µL Lipofectamine™ 2000 (L2000+CombiMag) were injected into tail vein. Prior to Magnetofection, a magnet was positioned onto the tumour surface for 15 min. Tumours were removed at 24, 48 and 72H post transfection and IGF-1R protein production was analysed. *From Wang et al. Biochem Biophys Res Commun 2011. 410:537-542 (10).*

The results show the efficiency of Magnetofectamine™ for targeted *in vivo* DNA delivery

Other results with Magnetofection™ *in vivo*

In vivo PolyMag-like – gene delivery

Transfection of heart and lung in mouse. Li and coll. have transfected mouse epicardial cells after systemic injection.



The authors compared the luciferase expression obtained in heart, left lung and right lung of mice after tail vein injection, both in presence and in absence of a magnet attached to the left epicardial surface. In the presence of magnet, the level of luciferase expression in the heart increased more than three orders of magnitude compared with control mice without implantation of an epicardial magnet. There were no significant luciferase expression variations from the right lung which has no direct influence by the magnetic field. *From Li et al, J. Magn. Magn. Mater., 2007; 311:336-341 (11).*

Others Magnetofection applications

Transfection of ischemic skin flaps with magnetobubbles. Holzbach and coll. investigated the Magnetofection™ potential of magnetic lipospheres containing DNA encoding VEGF on survival and perfusion of ischemic skin flaps. Magnetofection™ induced an increase in flap survival of 50% and an increase in flap perfusion. These results were comparable to those obtained with adenoviral transduction but presented superior safety profile (12).

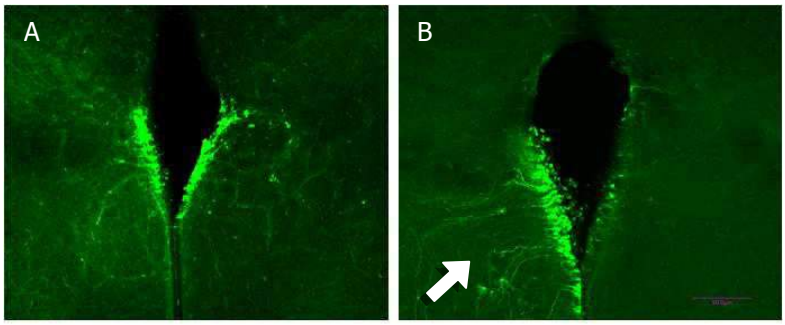
Transfection of lungs with magnetic aerosol droplets. In their study published in Nature Nanotechnology, Dames and coll. demonstrated for the first time the feasibility of a targeted delivery of magnetic droplets to the lung in an intact animal model. This approach overcomes the natural deposition mechanism of inhaled aerosol droplets in the lungs that only allows targeting on the central airways or lungs periphery, but not local regions in the lungs. A two-fold higher dose of plasmid DNA was found in the magnetized right lung than in the non-magnetized left lung. These results open a way to gene therapy by Magnetofection™ (13).

In vivo Magnetofection™ – Viral applications

Tissue targeting

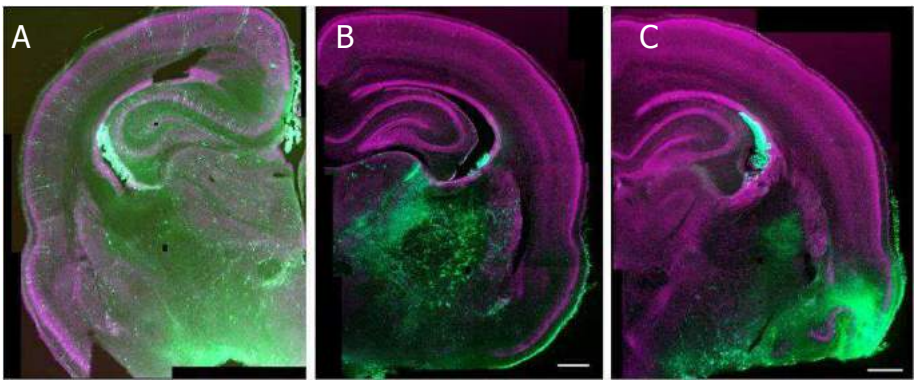
In vivo ViroMag – targeted viral infection icv

Infection of rat embryo brain with adenovirus. Sapet and coll. have shown that magnetic nanoparticles enhanced adenovirus transduction *in vivo* after intracerebroventricular injection (i.c.v). Moreover, infection could be confined and directed towards the magnetic field.



The uterine horns of pregnant rats [embryonic day 15] were exposed, and the third ventricle of each embryo was injected with Fast Green combined with Ad-GFP complexed to AdenoMag. When injected with AdenoMag, a magnet was applied for 30s following injection on one side of the embryo cranium. After injection, the uterine horns were replaced. Pictures show the third ventricle of E17 rat embryo (2 days post surgery). In brains non-exposed to magnet (A), the adenovirus-transduced cells are located on both side of the ventricle; cells are mainly restricted to this region indicating that the injection took place in this part of the brain. In brains exposed to magnetic field (B), the infected cells are located on one side of the ventricle due to the 30 s magnet-application represented by white arrow. *From Sapet et al. Pharm Res 2012; 29:1203-1218 (14).*

Infection of rat embryo brain with lentivirus. **ViroMag** magnetic nanoparticles associated with lentivirus have been used by Sapet and coll. to concentrate and target viral transduction in rat brain. Results showed a significant enhancement of gene expression with Magnetofection™ than with standard infection.

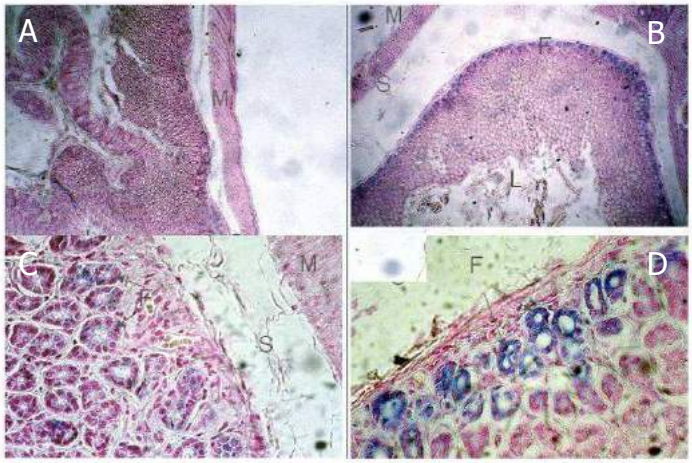


Brain sections at 8 days after lateral ventricular injection of 10^9 particles of GFP-lentivirus coupled with **ViroMag** into *in utero* rat embryos (E16) showed a diffuse GFP expression (in green) due to a widespread infection of neurons (A). The association of GFP-lentivirus with **ViroMag** induced a targeted local area as shown by the GFP expression in neurons lying under a magnet at the surface of the embryo skull (B). A more intense and restricted GFP-expression (C) was also observed when the magnet was positioned on the edge of the brain leading to an accumulation of viral particles and infected neurons in the focal area. *From Sapet et al. ; Drug Delivery Technol., 201010:24-29 (15).*

The results present the high efficiency of *In vivo* ViroMag for targeted delivery of viral vector after intracerebroventricular injection

In vivo ViroMag – adenoviral infection

Transduction in mouse stomach. Gene delivery and gene therapy in stomach are hampered by the extreme conditions of pH, the abundance of degradative enzymes, the presence of degraded nutriments and bacteria. In their study published in Gene Therapy, Scherer *et al.* successfully applied adenoviral vector and **ViroMag** complexes to the lumen of the stomach of mice for transducing the deep cells of the fundic glands. In this model, a permanent magnet was positioned under the stomach covering the area of the gastric fundus.



In the absence of a magnetic field yields, gene delivery occurred in only a few transfected cells (A,C), while exposure to a magnet for 20 min produces strong and widespread transgene expression (X-gal staining) in the crypts of the fundic glands 4 days after gene delivery (B,D). The adenoviral Magnetofection applied to the stomach produced strong staining of the crypts of fundic glands. *L, lumen; LP, lamina propria; F, fundic glands; S, submucosa; M, muscularis. 40. magnification (upper panel), 400. magnification (lower panel). From Scherer et al., Gene Therapy., 2002; 9:102-109 (3)*

The results show the efficiency of *In vivo* ViroMag for virus infection in tissue with harsh environment

In vivo ViroMag – lentiviral infection

Transduction in cardiovascular system. Viral assisted and targeted magnetic infection has also been established by Burdorf and coll. in a rat heart transplantation model. The authors injected lentiviral particles/**ViroMag** magnetic nanoparticles complexes into aortic root and placed a permanent magnet on the heart under cardioplegic arrest at 4°C for 30 min. Despite the hypothermic conditions and the time constraints, an efficient viral infection was clearly demonstrated in both endothelial cells and myocytes (16).
In addition, Hofmann and coll. used **CombiMag** magnetic nanoparticles mixed with a lentivirus to transduce endothelial cells of mice aorta in presence of physiological hydrodynamic forces (17).

The results show the efficiency of *In vivo* ViroMag for lentiviral transduction in hard-to-transfect conditions

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