Chapter

22

PLEIOTROPIC FUNCTIONS OF MAGNETIC NANOPARTICLES FOR *EX VIVO* GENE TRANSFER AND CELL TRANSPLANTATION THERAPY

Daisuke Kami[1*], Masashi Toyoda[2], Masatoshi Watanabe[3], and Satoshi Gojo[4]

[1] Department of Regenerative Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan
[2] Department of Vascular Medicine, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[3] Faculty of Engineering, Yokohama National University, Kanagawa, Japan
[4] Department of Regenerative Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan

*Corresponding author: dkami@koto.kpu-m.ac.jp

Contents

22.1. INTRODUCTION 549

22.2. NANOTECHNOLOGY AND MAGNETIC NANOPARTICLES 549

22.3. GENE TRANSFER USING MNPs 550

22.4. STRATEGIES FOR THE DEVELOPMENT OF A NEW METHOD FOR CELL TRANSPLANTATION THERAPY USING MNPs 552

22.5. FUTURE DEVELOPMENT 554

REFERENCES 555

22.1. INTRODUCTION

Magnetic nanoparticles (MNPs) are widely used in research and medical applications and have attained new levels of versatility and functionality. In this study, we determined the feasibility of traceable *ex vivo* gene transfer using MNPs instead of a multiple-step approach involving *in vitro* gene transfer, isolation of cells, and marking for *in vivo* tracing. This is a breakthrough technology that introduces new possibilities and a novel concept for cell transplantation therapy. In this chapter, we describe the development of this concept and its future potential.

22.2. NANOTECHNOLOGY AND MAGNETIC NANOPARTICLES

Nanotechnology is the manipulation and application of nano-sized materials. These methods have already been applied in various fields. Compared to micro-sized particles, nanoparticles (approximately 100 nm) that exhibit magnetic characteristics display higher fluidity and surface area as well as improved reaction efficiency. These particles also express magnetic properties and show easily controllable behaviours; therefore, they produce a strong effect in a limited space [1].

Divalent or trivalent iron oxide is the major material used in the formulation of magnetic nanoparticles (MNPs). Iron oxide presents low cell toxicity and has been used in the clinical field as a contrast agent for magnetic resonance imaging (MRI), which will be described later. MNPs have also been prepared using cobalt, manganese, nickel, and neodymium metal oxides; however, these display stronger cytotoxicity than iron oxide MNPs, and thus need to be coated [2]. Coating substances are generally selected to reduce the cytotoxicity of magnetic particles. In many cases, dispersing agents are used as coating substances to provide additional functionality to the nanoparticles.

Researchers are currently attempting to add functionality to the surface of MNPs [2]. For example, particles coated with dispersants, which enhance dispersibility, could be unmobilised under a magnetic field. Antibodies can also be coupled to particle surfaces, allowing the effective acquisition of a target *via* interactions between the coupled nanoparticles and proteins, bacteria, or cells with epitopes for the antibody that exclusively express the specific protein (magnetic-activated cell sorting, immunoprecipitation, and magneto-microfluidics) [3]. In addition, pharmaceutical agents, such as anticancer drugs, could be coupled with MNPs and administered at a targeted site at the minimum required dosage. These complexes could then be localised under a

magnetic field, thereby reducing adverse effects to the body. MNPs could serve as an effective drug delivery system [4].

In addition, the fine vibrations caused by exposure of MNPs to an alternating magnetic field result in the generation of heat. This phenomenon, called hyperthermia, could be applied to the treatment of cancer. Recently, a system has been devised wherein MNPs are concentrated within the cancer *via* neoangiogenic blood vessels, and heat is generated using an alternating magnetic field [5].

MRI contrast agents using iron oxide have been commercially available since the late 1990s. Various diagnostic utilities of MNPs have previously been reported, and these particles are highly reliable contrast agents for normal use. MRI, using iron oxide-based contrast agents, is generally the first choice as the most effective and non-invasive technique for the diagnosis of metastatic liver cancer. Moreover, recent advances in MRI technology have given rise to new prospects for MRI use (in combination with other analytical methods).

As mentioned above, MNPs can be applied in various fields and potential for use in the medical care industry in the near future.

In this chapter, we report a novel cell transplantation treatment strategy focusing on gene transfer using MNPs.

22.3. GENE TRANSFER USING MNPs

One of the main biological applications of MNPs is their use in a gene transfer method called magnetofection [6], in which nucleic acids such as DNA (plasmids) and RNA can be transferred into cells. Such nucleic acid transfer is an important tool that is routinely used in current life science research, such as for the control of target gene expression and cell labeling. In recent years, gene transfer technology has been successfully used in the production of induced pluripotent stem cells (iPSCs) [7,8] and in Cas nuclease RNA-guided genome editing (CRISPR) [9] or transcription activator-like effector nuclease (TALEN) [10] editing, thus suggesting a further increase in its importance in the near future.

Currently, there exist three principal methods for gene transfer: (1) viral vectors, such as recombinant retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses, to deliver genomic materials into cells; (2) electroporation to disturb cell membranes by electrical stimulation and promote the passive transfer of genes of interest into cells; and (3) chemical reagents such as cationic polymers to encircle nucleic acids, fuse with cell membranes, and release them into the cytosol. Among these, the method utilising a chemical reagent is suitable for clinical applications owing to its relatively low cytotoxicity with negligible genomic incorporation. However, the gene transfer efficiency of chemical reagents is generally lower than that of

other methods. Therefore, the improvement of transfer efficiency using the reagents has been highly anticipated.

We attempted to improve the gene transfer efficiency of the transfection reagent by utilising MNPs as nucleic acid carrier agents. Divalent and trivalent iron, cobalt, manganese, nickel, and neodymium are currently available MNPs. Among these, iron oxide is used as an MRI contrast agent and is known to display a low cytotoxicity. Therefore, we used iron oxide as the nucleic acid carrier and the cationic polymer poly(ethyleneimine) (PEI) as the dispersing agent for iron oxide. PEI is a well-characterised polymer that has been used as a gene transfer reagent. Because of the commercial availability of PEI with various molecular structures, modifications, and molecular weights, the specific form of PEI can be selected according to the application. We chose deacylated PEI (PEI max, Polysciences, Inc., Warrington, PA, USA.), which displayed lower cytotoxicity and a linear form, to coat the iron oxide. This type of PEI was also more cationic than typical PEI. The MNPs composed of iron oxide and deacylated PEI display high dispersibility and cohesiveness under the magnetic field; this enables the construction of a superior magnetofection system (Figure 1, Tables 1 & 2). In addition, the low toxicity of these nanoparticles allow for the simultaneous introduction of multiple plasmids [11].

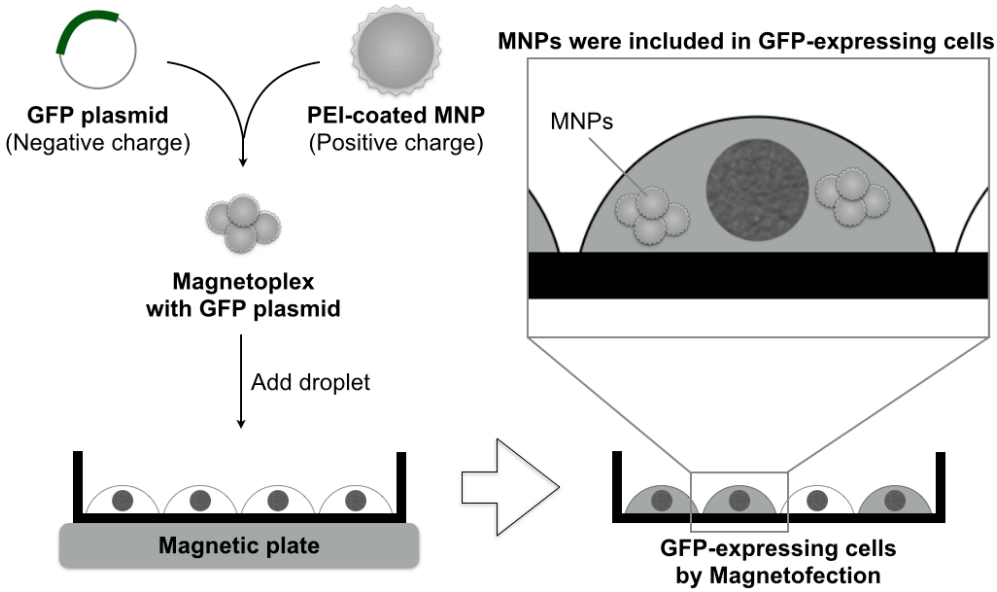

Figure 1. Diagrammatic illustration of magnetofection

Table 1. Reaction mixture of magnetofection for 6 well plate

Plasmids (1 μg/μl)	1.0 μl/well
PEI-coated MNPs (50 μg MNPs/ml)	7.5 μl/well
Deionised water or Opti-MEM*	41.5 μl/well
Total **	50.0 μl/well

* Life Technologies, Inc.

** Mixtures were reacted for 15 min at room temperature

Table 2. Transfection efficiency of our magnetofection method

Cell line	Species	Description	Transfection efficiency	References
P19CL6	Mouse	Embryonic carcinoma	81 %	[12]
MEF	Mouse	Embryonic fibroblast	9 %	[13]
HeLa	Human	Cervical cancer cell	40 %	[14]
TIG-1	Human	Fetal lung fibroblast	--	[11]

The gene transfer efficiency of this magnetofection system depends on the size of the magnetoplex, which is comprised of MNPs and plasmids. Cationic polymer PEI-coated MNPs (positive charge) and nucleic acids (negative charge) are electrodynamically coupled to form the magnetoplex complex [14]. The size of the magnetoplex complex varies according to the amount of MNPs used. A larger quantity of MNPs results in a larger number of nucleic acids that are coupled and a larger magnetoplex. The gene transfer of MNPs is dependent on endocytosis, the mechanism by which the magnetoplex enters the cell. The larger the size of a magnetoplex, the poorer the efficiency of gene transfer [14]. This trade-off is quite important to establish an optimal condition for magnetofection, which depends upon the host cells.

22.4. STRATEGIES FOR THE DEVELOPMENT OF A NEW METHOD FOR CELL TRANSPLANTATION THERAPY USING MNPs

Based on the properties described above, we reported a concept that could assist in the development of a new method for cell transplantation therapy using magnetofection in 2014 [13]. The transfection of genes into cells using magnetofection involves the capture of MNPs within the cells (Figure 1). A chronological quantitative measurement of the residual amounts of nanoparticles within the cells using an inductively coupled plasma mass spectrometer demonstrated no significant changes in the number of MNPs per cell over a two-week period. In addition, as the cells detached by trypsinisation

reacted to the magnetic force, it was possible to control their dynamics within the solution. Based on these findings, we hypothesised and attempted to demonstrate the separation and purification of magnetofection-treated gene transfected cells using magnetic force, and the tracing of these cells *in vivo* using MRI after grafting into mice. This strategy can be effectively applied to (1) highly efficient gene transfer, (2) the separation and purification of cells by magnetic force (*in vitro* cell separation), and (3) imaging of the transplanted cells *in vivo* using MRI (Figure 2). The pleiotropic roles of MNPs are easily applied to a single system for cell transplantation therapy.

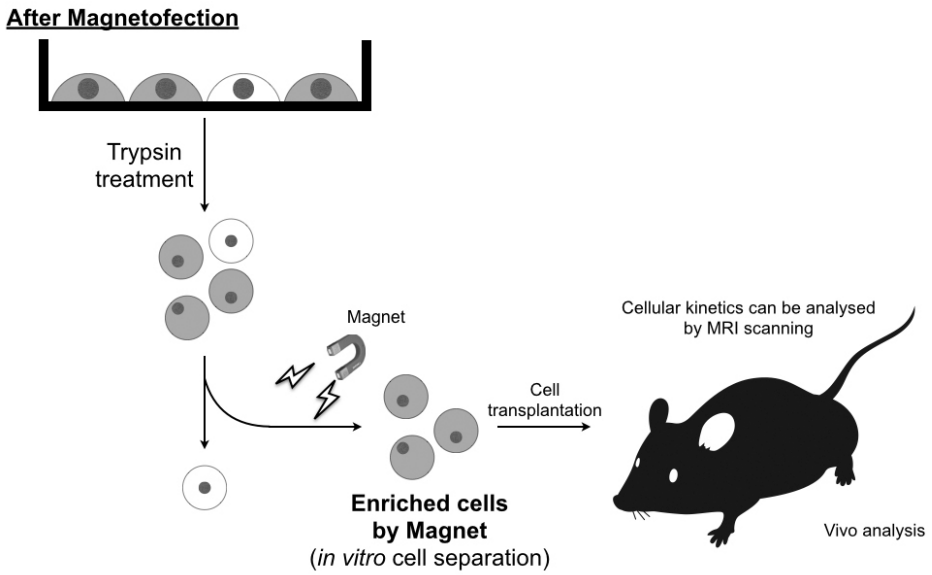

Figure 2. Diagrammatic illustration of the new strategy for cell transplantation therapy using MNPs

Because magnetofection introduces a large amount of nucleic acids into cells, the cells are subjected to a high degree of cytotoxicity. However, it has been discovered that MNPs themselves exhibit almost no cytotoxicity, and that the level of cytotoxicity increases with the quantity of nucleic acids in a dose-dependent manner. Future research should aim to optimize the cytotoxicity and gene transfer efficiency of these nanoparticles. According to our current preliminary data, a high gene transfer efficiency and low cytotoxicity can be achieved using approximately half the conventional nucleic acid quantity. Because a high transfer efficiency can be achieved using a small quantity of

nucleic acids, there is a low chance of nucleic acid insertion into the genomic sequence of host cells, which is an advantage for cell transplantation. These results will be summarised in the future.

22.5. FUTURE DEVELOPMENT

Cell transplantation therapy is currently being carried out worldwide, with many research groups utilising autologous somatic stem cells [15]. A major reason for the transplantation of cells into a patient following *in vitro* cell culture and proliferation is to bypass ethical issues and immune rejection of cell transplantation [16]. Moreover, the secretion of paracrine cytokines has been suggested to be the major mechanism influencing the efficacy of the transplanted cells. However, transplanted cells display individual secretory properties with respect to cytokines, exosomes, microRNA, and so on [17,18]. There also exist cases with few targeted cytokines or low secretion of exosomes and microRNA, since it is quite difficult to verify the quality of donor cells. This would cause variation in the overall therapeutic effect. In addition, owing to the difficulties associated with cell tracing after transplantation, it would not be possible to determine the amount of time that cells would remain in a required site.

The novel concept of cell transplantation therapy reported in this chapter offers a solution to these problems. By introducing targeted nucleic acids into cells using MNPs, cells with stable characteristics can be produced. Furthermore, transfected cells can be purified using magnetic force. Since the dynamics of these purified cells can be observed non-invasively using MRI even after transplantation into the target organs, it would be possible to assess the resulting cell behaviour. This allows the standardisation and better management of cell transplantation therapy, a process for which quality control was previously thought to be difficult. This strategy is a good example of theranostics using nanotechnology.

It is likely that the modification of nanomaterials using various techniques can provide added value to cell transplantation therapy in the future. These techniques may represent a breakthrough, reviving the currently stagnant cell transplantation therapy field.

REFERENCES

1. J. Leszczynski. *Nat. Nanotechnol.* **5**(9) (2010) 633–634.
2. X.R. Xia, N.A. Monteiro-Riviere, J.E. Riviere. *Nat. Nanotechnol.* **5**(9) (2010) 671–675.
3. J.H. Kang, M. Super, C.W. Yung, R.M. Cooper, K. Domansky, A.R. Graveline, T. Mammoto, J.B. Berthet, H. Tobin, M.J. Cartwright, A.L. Watters, M. Rottman, A. Waterhouse, A. Mammoto, N. Gamini, M.J. Rodas, A. Kole, A. Jiang, T.M. Valentin, A. Diaz, K. Takahashi, D.E. Ingber. *Nat. Med.* **20**(10) (2014) 1211–1216.
4. A.Z. Wilczewska, K. Niemirowicz, K.H. Markiewicz, H. Car. *Pharmacol. Rep.* **64**(5) (2012) 1020–1037.
5. M. Lin, J. Huang, M. Sha. *J. Nanosci. Nanotechnol*. **14**(1) (2014) 792–802.
6. F. Scherer, M. Anton, U. Schillinger, J. Henke, C. Bergemann, A. Kruger, B. Gansbacher, C. Plank. *Gene Ther.* **9**(2) (2002) 102–109.
7. K. Takahashi, S. Yamanaka. *Cell* **126**(4) (2006) 663–676.
8. K. Okita, H. Hong, K. Takahashi, S. Yamanaka. *Nat. Protoc.* **5**(3) (2010) 418–428.
9. M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna, E. Charpentier. *Science* **337**(6096) (2012) 816–821.
10. J. Boch. *Nat. Biotechnol*. **29**(2) (2011) 135–136.
11. D. Kami, S. Takeda, Y. Itakura, S. Gojo, M. Watanabe, M. Toyoda. *Int. J. Mol. Sci.* **12**(6) (2011) 3705–3722.
12. D. Kami, S. Takeda, H. Makino, M. Toyoda, Y. Itakura, S. Gojo, S. Kyo, A. Umezawa, M. Watanabe. *J. Artif. Organs* **14**(3) (2011) 215–222.
13. D. Kami, T. Kitani, T. Kishida, O. Mazda, M. Toyoda, A. Tomitaka, S. Ota, R. Ishii, Y. Takemura, M. Watanabe, A. Umezava, S. Gojo. *Nanomedicine* **10**(6) (2014) 1165–1174.
14. S. Ota, Y. Takahashi, A. Tomitaka, T. Yamada, D. Kami, M. Watanabe, Y. Takemura. *J. Nanopart. Res.* **15**(3) (2013) 1653–1664.
15. D. Kami, S. Gojo, A. Umezawa. *Rinsho Ketsueki* **54**(5) (2013) 436–443.
16. S. Gojo, A. Umezawa, *Hum. Cell* **16**(1) (2003) 23–30.
17. P.D. Robbins, A.E. Morelli. *Nat. Rev. Immunol.* **14**(3) (2014) 195–208.
18. A.R. Williams, J.M. Hare. *Circ. Res.* **109**(8) (2011) 923–940.