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Magnetofection: A Magical Technique for Effective Gene Transfer Using Magnetic Nanoparticles

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Abstract

Gene therapy is a type of therapy that works by turning off disease-causing or malfunctioning genes and delivering a specific gene to the body to treat the disease. Delivering a therapeutic gene to targeted cells remains a limitation of gene transfer. Gene transfer is therefore an important part of gene therapy. Gene delivery systems are generally divided into viral-based and non-viral-based systems. Among many nanostructures, nanoparticles are widely used as vectors for non-viral gene transfer. Magnetic nanoparticles (MNPs) have been widely used in the biomedical field in recent years due to their unique magnetic properties. In principle, their charge and size make MNPs suitable for reaching the target site. Furthermore, the high surface area/volume ratio makes MNPs ideal for gene transfer. One of the main methods of using MNPs for gene transfer is magnetofection. In this method, DNA and MNPs are combined in a buffer containing salt to form a complex called magnetofectin. This complex is allowed to penetrate cells under the influence of a magnetic field. DNA, which is negatively charged, needs to be modified to pass through the negatively charged cell membrane, to form complexes with MNPs, and to increase its stability and biocompatibility. For this purpose, commonly used polymers such as PEI (e.g., amphiphilic poly(L-lysine), polyamidoamines (PAAs), and PEG) are used as gene carriers. In addition, MNPs and polymers such as PEI aid the endosomal escape of DNA. This mini review summarizes the specific gene transfection (magnetofection) of magnetic particles during all dynamic processes of gene transfer (nanoparticle synthesis, gene binding, cellular uptake, endosomal escape, and in vivo targeting).

Keywords: Magnetic nanoparticles, magnetofection, gene therapy, biomedical applications, combination therapy

Introduction

Gene therapy is a strategy that uses genetic material to modify cells (*in vitro* or *in vivo*) for therapeutic purposes. Genes are not able to enter cells passively. In order to transfer them across the hydrophobic and negatively charged cell membranes and safely deliver them to the target region with great efficiency, an appropriate vector or carrier system is required. Insufficient gene

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transfer vectors that are safe, effective, and unique to a certain cell are the cause of low transfection efficiency (1). The efficiency of transfection depends on the efficient transfer of nucleic acids into cells. Therefore, in gene therapy, it is necessary to select a suitable carrier to deliver the target gene to the cell in a specific way. There are several potential barriers to decreasing the efficiency of gene delivery, such as degradation of nucleic acids by extracellular enzymes, inability of vectors to enter target cells, problems in intracellular transfer of nucleotides from endosome to lysosome and escape from the endosome, releasing of nucleic acids from carriers, and inability to enter the cellular nucleus (2–4). Viral or non-viral vectors are used to deliver the exogenous gene into the cell. In viral vectors, any nucleic acid can be loaded into differently designed viruses and used as a carrier to deliver the desired gene into cells. However, the disadvantages are that they are expensive and complex to prepare, cause an unexpected inflammatory response, and interfere with the immune system. Non-viral vectors are safer, as they show less cytotoxicity and immunogenicity (1).

Magnetic nanoparticles (MNPs), one of the non-viral vectors, are used in biotechnology as drug or gene carriers in the diagnosis and treatment of diseases. The exogenous gene is bound to MNPs and delivered to the cells with the help of a magnet. This technique is defined as magnetofection. This method is considered safer than viral vectors (5,6). Magnetofection is a non-viral gene delivery system that is effective in vitro and in vivo (7,8). In this method, gene transfer is usually performed using polymer-coated and biodegradable iron oxide MNPs (9).

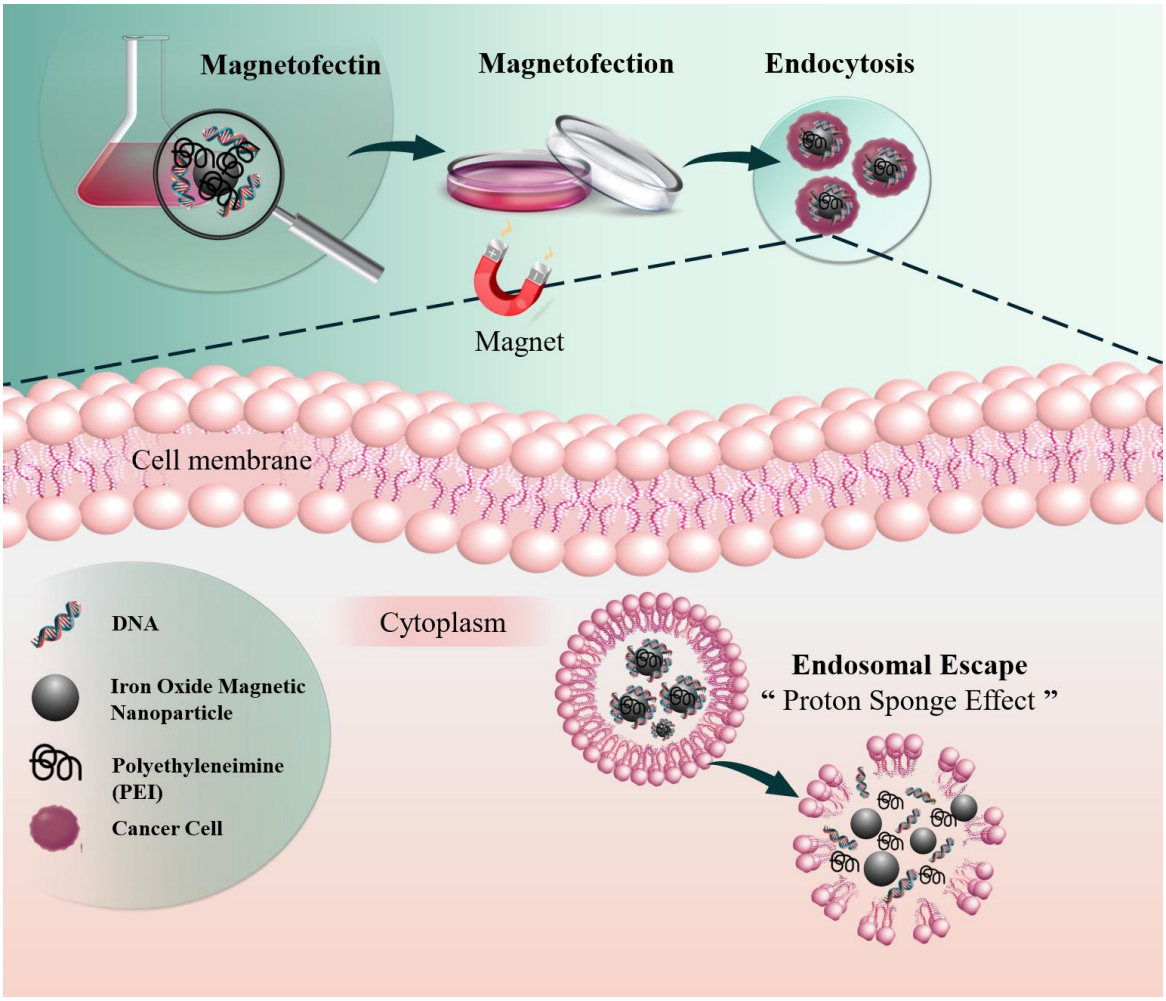

Figure 1. The summary of this study.

2. Gene transfer using magnetic nanoparticles: The distinct magnetic characteristics of MNPs have made them popular in the biomedical industry in recent years. Technically, MNPs can reach the target cells or tissues because of their size and surface charge. Surface functionalization is one way to increase the biocompatibility of MNPs. Furthermore, the high surface area/volume ratio of MNPs makes them very advantageous for gene transfer. The delivery of large, fragile DNA to the cell nucleus without its degradation by nucleases is a prerequisite for the success of gene therapy (1).

In gene transfer by magnetofection, MNPs are brought together with vector DNA (magnetofectin). Magnetofectins are delivered to the target cell under the influence of a magnetic field with the help of a magnet. This method is known to increase transfection efficiency even in cells that are difficult to transfect under in vitro conditions (10)(11). Successfully delivered pCMV-Azu-GFP recombinant DNA to MCF-10A cells by magnetofection method in a study. In the study, silica-coated iron oxide nanoparticles formed magnetofectin complexes by combining PEI and recombinant DNA and successfully transfected the complex into the cell with the help of a magnet. They confirmed protein expression after magnetofection by confocal microscope imaging and western blot analysis.

3. Gene binding and cellular uptake: Electrostatic adsorption is a straightforward method of attaching negatively charged genes to MNPs by covering their surface with cationic chemicals. This is one of the simplest techniques of DNA binding in gene transfer. MNPs can be coated with cationic molecules such as PEI, poly-L-argine, poly-L-lysene, and chitosan to ensure effective gene delivery. Magnetofection is more effective when the MNPs' surface is modified since it improves the DNA binding capabilities. (11,12). The PEI attaches the DNA because it is positively charged at physiological pH. Furthermore, due to the buffering activity of PEI (the proton sponge effect), PEI disrupts endosomes, allowing DNA to escape from the endosome without using any additives to disrupt endosomes. In addition, the magnetic field enables faster transfer of DNA to the target cell and facilitates cellular uptake (4,13,14). The magnetofection process is briefly described as follows: In a salt buffer, plasmid DNA and MNPs form colloid aggregates. When such particles are mixed with naked DNA, lipoplexes, or polyplexes in a salt-containing buffer, they bind or aggregate these compounds. Magnetofectin, consisting of MNP, DNA, and PEI, is applied to the cells, and the complex is taken up into the cell by a magnet. It is assumed that almost every cell will be exposed to the plasmid DNA as a result of the magnetofection process, enabling the transfection of cells (11,12).

4. Endosomal escape and in vivo targeting: Materials that have been endocytosed converge with lysosomes in the cellular environment, where proteolytic enzymes accelerate their destruction. In the magnetofection, a potent gene delivery technique, the DNA/MNP complex is internalized via endocytosis. However, it is essential to avoid lysosomes breaking down the magnetofectins. This problem is solved by using cationic gene carrier polymers, such as PEI, and taking advantage of the "proton sponge effect". These polymers serve to buffer the endosomal vesicle subsequent to their complexation with DNA upon cellular uptake. Consequently, endosomal lysis and swelling ensue, thereby shielding the targeted DNA from lysosomal degradation until its successful transfer to the desired cellular destination (15).

Superparamagnetic iron oxide nanoparticles (SPIONs) coated with polyacrylic acid (PAA, 8 kDa) and PEI (25 kDa) (SPIONs-PAA-PEI) are safe and effective for magnetofection of tumor cells in mice (16). The SPIONs-PAA was incubated with PEI overnight before administration. pDNAIL-12, a plasmid encoding the immunostimulatory cytokine interleukin 12 (IL-12), was used to stimulate an immune response against the tumor. The administration of the magnetofectin

complex resulted in a 0–1 day delay in tumor growth without a magnetic field and a 7–9-day delay with a magnetic field on day 10 following the last injection.

MicroRNA-encoding plasmid DNA was delivered using chondrosulfate-PEI copolymer -coated (CP) SPIONs, an effective magneto-gene carrier (17). The magnetofection gene carrier was prepared by complexation through electrostatic interactions between CP and self-assembled poly(acrylic acid)-bonded SPIONs (named CPIO). In nude mice with U87-xenografted tumors in the right and left hindlimb areas, the bio-distribution of CPIO/Cy5-DNA was examined in order to assess the in vivo magneto-induced uptake of magnetoplex. The signals in the two tumors were finally analyzed after 48 hours, and it was discovered that the tumor with the magnet had higher permeability and retention (EPR) effect and a noticeably brighter signal(18). Functionalized SPIONs with an active catechol ester (CafPFP) and covalently bound PEI to their surface. They reported that when plasmid DNA (pDNA) and SPIONs were mixed up to 2.5% pDNA/Fe weight ratio, 95% of the plasmid DNA was strongly bound to the SPION surface. A375-M cells were treated with the magnetofectin complex that was obtained at various doses. According to their findings, the particles and incubation durations examined using the pDNA-SPION system did not exhibit any harmful effects on the cells.

PEG-modified-PLGA-PEI MNP was used to successfully transfect primary hippocampus neurons in a work by Cui et al. (19). According to their findings, mice's hippocampal nanoneurotoxicity was also markedly decreased by PEG-modified-PLGA-PEI MNP.

Conclusion

The effectiveness of gene delivery mechanisms remains a critical determinant of therapeutic success. Challenges such as cellular uptake, endosomal entrapment, and in vivo targeting are essential for achieving significant clinical outcomes. Biocompatibility, surface features that may be customized, and the ability to be manipulated with a temporary magnet make magnetic nanoparticles (MNPs) an adaptable platform.

Magnetofection is the process of effectively delivering genetic material into target cells by making use of the special potentials of MNPs. Through electrostatic adsorption and custom-made surface modifications with cationic agents, MNPs serve as efficient carriers, ensuring the protected binding and internalization of genetic cargo. The strategic utilization of polymers such as PEI, with its "proton sponge effect," further enhances endosomal escape, safeguarding the integrity of the genetic materials during intracellular transition. Magnetofection holds promise for revolutionizing gene therapy to enhance targeting precision, improve transfection efficiency, and mitigate off-target effects.

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Methodology: KS, ŞS, Bİ, ŞYA
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