WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 7, 469-487. Review Article ISSN 2277– 7105

MAGNET-GUIDED NANOVECTORS AS AGENTS FOR MAGNETOFECTION IN THERAPEUTIC MANAGEMENT OF SOLID TUMORS

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Article Received on
04 May 2016,
Revised on 25 May 2016,
Accepted on 16 June 2016
DOI: 10.20959/wjpr20167-6541

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ABSTRACT

Efficient delivery and accumulation of gene into solid tumors via physical and chemical methods is restricted by lack of tissue selectivity, host cell tropism, immunogenicity and several other factors. Magnetofection shows great promise in external magnet-guided delivery of different types of nucleic acids, non-viral transfection systems and viruses via nanoparticles in therapeutic management of solid tumors. These nanovectors are endocytosed by the target cells leading to high concentration of vector accumulation and retention of genetic payload at significantly higher level inside tumor tissues. Transfection efficiency by magnetofection can be influenced by judicious selection of cells of proper type and of good health and viability, in addition to choice of ideal polymer. The technique has been exploited for gene delivery to internal organs and to central nervous system. Due to its several advantages, it is employed as an ideal research tool for genetic engineering. Magnetofection, therefore, has the potential to turn the challenge of gene therapy in vivo into a new frontier for treatment of solid tumors.

KEYWORDS: solid tumor, gene therapy, viral and non-viral vectors, magnetofection.

1. SOLID TUMORS

Solid tumors are abnormal masses of tissues, commonly referred to as “lumps” that a person (or a doctor) can see or feel, like a lump in a breast or a polyp in a colon.[1] They usually do not contain cysts or liquid areas. They may be benign (not cancerous) or malignant

(cancerous). It has been reported that about 80% of cancer comes from solid tumors**.** On the basis of their occurrence, solid tumors can be classified as

- **Sarcomas**

Cancers arising from connective or supporting tissues, for example bone or muscle.

- **Carcinomas**

Cancers arising from the body's glandular cells and epithelial cells, which line body tissues.

- **Lymphomas**

Cancers of the lymphoid organs such as the lymph nodes, spleen, and thymus, which produce and store infection-fighting cells.[2]

The estimation of the cancer incidence in major states of India in the year 2016 is projected in Figure 1.[3]

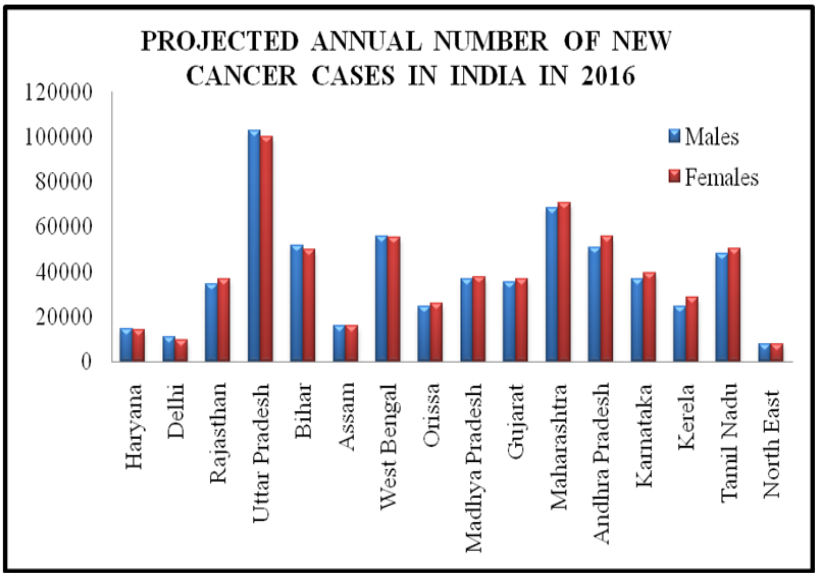


Figure 1: Projected annual number of new cancer cases in India in 2016

The estimated number of new cancer cases in India by the year 2026 is projected in Figure 2. The results reveal that during the year 2011 an estimated 1,193,426 (1.19 million) persons developed cancer and by the year 2026, the estimates of cancer incidence might increase to 1,869,983 (1.87 million). It has been observed that new cancer cases in female are higher as compared to males at all time periods.[3]

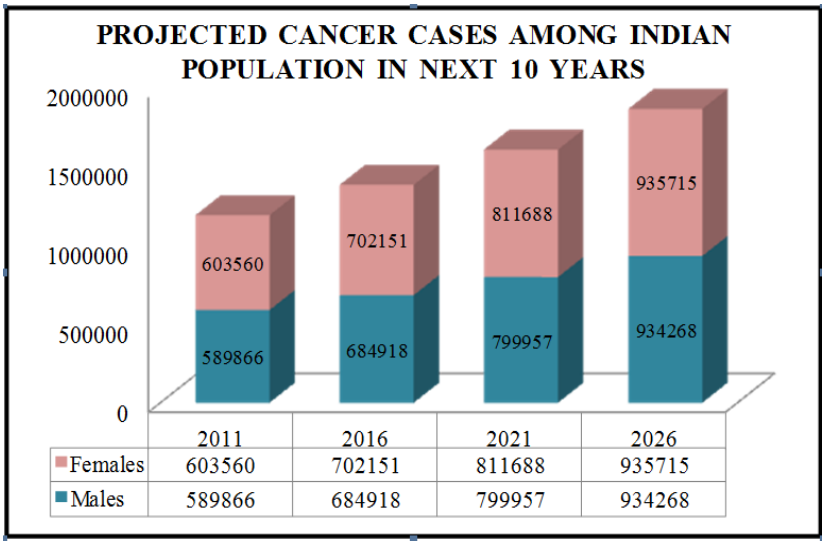


Figure 2: Projected cancer cases among Indian population in next 10 years

2. TREATMENT STRATEGIES OF SOLID TUMORS

There are various treatment strategies for solid tumors that can be broadly classified into two main categories

A. Non-gene therapy

i. Surgery

More patients are cured of cancer with surgery, i.e., by excision of tumor.

ii. Radiation

It is used for the treatment of localized solid tumors when surgery is not appropriate or feasible (e.g., inoperable lung cancer). Radiation injury to the lung may produce pneumonitis and pulmonary fibrosis causing hypoxia and dyspnea. Irradiation of salivary glands may cause dryness of mouth or painful mouth sores.[4]

iii. Chemotherapy

Treatment of malignant cells by antineoplastic chemotherapeutic agents has a tendency to cause alopecia, stomatitis, myelosuppression, carcinogenicity in several instances. Moreover, poor vasculature of solid tumor masses poses a barrier to drug penetration and delivery. Drug-resistant cells may arise due to prolonged therapy with chemotherapeutic agents.[5]

iv. Hormonal therapy

It is effective in cancer dependent on hormones. For example, some anti-estrogen, anti-androgenic or corticosteroids can be used for the treatment breast cancer, metastatic prostate

cancer and leukemia respectively. Hormone therapy may cause immediate and sustained suppression of testosterone.[6]

v. Stem cell transplantation

In stem cell transplantation, in order to achieve a higher rate of cancer cell killing, higher than normal doses of chemotherapy drugs need to be given, which can cause side effects like graft versus host disease (GVHD), low red blood cell count, organ damage, cataract, etc.[2,7]

B. Gene therapy

Gene therapy is selective delivery of gene or gene product to a specific cell/tissue to correct the loss of function caused by mutation or replace an "abnormal" disease-causing gene with minimal toxicity. It is a unique approach to treat both inherited and acquired diseases.[8] There are three primary groups of genes which when mutated, allow cells to grow rapidly and are responsible for cancer

a. Oncogenes

They promote cell proliferation and stop cell death, at times resulting in cancer. *HER2 and t*he *ras* family of genes are specialized protein that controls cancer growth and spread.

b. Tumor suppressor genes

They limit the development and/or growth of tumors. On mutation, it may fail to prevent a cancer from growing. *BRCA1*, *BRCA2*, and *p53* are examples of tumor suppressor genes.

c. DNA mismatch repair genes

These genes maintain integrity of the genome and the fidelity of information transfer from one generation of cells to the next; loss of function of DNA mismatch repair genes could make a cell error-prone.[9]

3. APPROACHES TO GENE THERAPY AND GENE DELIVERY

Since, in gene therapy a "normal" gene needs to be inserted into the genome to replace an "abnormal" disease-causing gene or a mutated gene, a carrier molecule called a vector is required to deliver therapeutic gene material and its associated regulatory elements into the nucleus.

3.1. Vectors – Carriers of gene therapy

Genes that help in transmission or get segregated during meiosis cannot be directly inserted into a person's cell. They are transported to the cell using carriers or DNA delivery vehicles

which are known as vectors. After reaching the target cell, the vector delivers the genetic material to the nucleus where it gets integrated into DNA and corrects the defective or mutated gene.

Vectors can be of two types

a) Viral vectors

b) Non-viral vectors

However, choosing the most suitable vector for treating any genetic disorder and ensuring high vector concentration and accumulation inside tumors is a challenge in gene therapy. To be successful, a vector must,

a) Target the right cell
b) Integrate the genes in the cell
c) Activate the gene
d) Avoid harmful side effects.[10]

3.1.1. Viral vectors

Viruses have evolved a way of encapsulating and delivering their genes to human cells thereby inducing pathogenicity. The delivery of genes by a virus is known as transduction and the infected cells are said to be transduced. Scientists have manipulated the viral genome by removing the disease-causing genes and inserting the therapeutic ones. These viruses bind to their hosts and introduce their genetic material by recognising the cell and altering the cell's DNA permanently or temporarily. Types of viruses used in gene therapy are retroviruses, adenoviruses, adeno-associated viruses and herpes simplex virus. Most of the viruses are double-stranded ones, except adeno-associated viruses. Retroviruses interfere with the natural biological processes, possibly leading to other diseases due to increased immunogenicity and cytotoxicity.[11]

Among all the vectors, adenoviruses as viral vectors are very advantageous for transduction of different types of cells used in basic research because they can be produced in high titers, and owing to their very efficient nuclear entry mechanism and low pathogenicity for humans.[12]

3.1.2. Non-viral vectors: Although, viruses are known to deliver genetic material effectively into a patient's cells, some genes may be too big to fit into some viruses as viral

vectors carry only a limited amount of genetic material. Receptor dependent host tropism of adenoviral vectors, short-lived therapeutic effects of adenoviruses limit their utility in gene therapy.[13] But advancement in efficiency, specificity, gene expression duration and safety has led to an increased number of non-viral vector products entering clinical trials. Non-viral vectors have drawn significant attention due to reduced immunotoxicity.

Non-viral vectors are generally used to transfer different types of nucleic acids, such as, small DNA (Oligodeoxynucleotides) or related molecules synthesized chemically, large DNA molecules (Plasmid DNA, DNA), RNA (Ribozymes, Si RNA, m RNA).[14]

3.2. Methods of gene delivery with non-viral vectors

3.2.1. Injection of naked DNA

Naked DNA is not specific for any cell type. Plasmid DNA will not integrate into the host cell's genome, unless it is specifically engineered to do so. Clinical trials carried out on intramuscular injection of a naked DNA plasmid found the expression to be very low in comparison to other methods of transfection.[13]

3.2.2. Physical methods to enhance delivery

Transferring of genetic material by physical methods employs physical force to destroy the integrity of the membrane barrier of the cells thus facilitating intracellular delivery of the genetic material. Various physical methods of gene delivery by non-viral vectors highlighting their applications and disadvantages are tabulated in Table 1.[13,15]

Table 1: Physical methods of gene delivery by non-viral vectors

METHOD	APPLICATION	DISADVANTAGE
i. Microinjection	Sample of interest is injected into the cell nucleus or cytoplasm of adherent cells	• Requires lengthy training period until reproducible results are obtained. • Only a few cells (100-200) can be injected in one experiment. • Not applicable to all cell types. • Cultures that grow in suspension and adherent cells that have only small volume nuclei or cytoplasm are more difficult to use.
ii. Electroporation	Based on the application of controlled, pulsed electric fields through which the target molecule enters the cell	• It may cause cell rupture or damage if pulses are of the wrong length or intensity and some pores may become too large or

		fail to close. • Transport of material is relatively non specific, may cause ion imbalance leading to cell death.
iii. Gene gun	DNA-particle complex is put on the top location of target tissue in a vacuum condition and accelerated by powerful shot to the tissue. The DNA is then effectively introduced into the target cells. It shoots DNA coated gold particles into the cell using high pressure gas.	• Transformation efficiency is low. • May cause damage to cells or tissues.
iv. Sonoporation	Involves the use of ultrasound assisted by encapsulated micro-bubbles (EMB) that can make cell membranes temporarily open and deliver macromolecules into cells. Ultrasound produces cavitation activity	Transformation efficiency is low
v. Hydrodynamic delivery	It causes transient pores to open in the cell membrane, and allow the entry of DNA into the cytoplasm and closes within 10 minutes of injection.	Application is limited to liver, muscle and kidney.

3.2.3. Chemical methods to enhance delivery

Chemical vectors are known to form condensed complex with therapeutic gene to protect them from nucleases and other blood components. Some of the chemical non-viral methods of gene delivery are

a. Oligonucleotides

b. Lipoplexes

c. Polymersomes

d. Polyplexes

e. Dendrimers

f. Inorganic Nanoparticles

g. Cell-penetrating peptides.[14,16]

Comparison of different physical and chemical approaches for gene therapy reveals interesting information. Most of the physical methods of gene delivery fail to achieve efficient transduction especially to hypoxic centre of tumor. Moreover, some of the methods are tissue-specific whereas, others are relatively non-specific. Accumulation of gene vectors

in target tissues depends on natural host cell tropism and biophysical properties. Tissue selectivity can be improved by engineering surface proteins of viral vectors, or coupling targeting ligands to viral as well as nonviral vectors. This technique of targeting cannot achieve rapid and specific accumulation of active vectors in target tissues.[15]

Among the chemical methods of gene delivery, some of the vectors show low insolubility under physiological pH condition and low transfection efficiency with relatively high cytotoxicity. Polymeric carriers like polyethyleneimine (PEI) show bad biodegradability.[16] Their transfection efficiency and toxicity depends on its molecular weight (MW), configuration and the charge ratio of polymer to DNA used. Several studies have shown that polymers with low to medium MW (5,000–25,000 Da) are more efficient and less toxic as compared to high MW PEI (greater than 25,000 Da) that are toxic to cells.[17] For cationic lipids the presence of the bulky polyethylene glycol (PEG) moiety on the surface prevents an intimate interaction between lipoplexes and cell membrane reducing the overall transfection efficiency. Acute toxicity and short duration of gene expression is a major disadvantage for practical use of lipoplex-mediated transfection.[16]

From above discussion on various physical and chemical methods of gene delivery by non-viral vectors, it is evident that almost all of them suffer from some drawbacks. To overcome some of these disadvantages, scientists have expanded the scope of magnetic drug targeting in delivery of gene vectors into cells and tissues. Basically, it consists of coupling of therapeutic gene to magnetic nanoparticle and can be used for gene delivery and hence, gene therapy with both viral and non-viral vectors in cancer treatment.[18]

4. MAGNETOFECTION

MAGNET GUIDED NANOVECTORS FOR GENE DELIVERY

Magnetofection is the method of transfection in which nucleic acids, either 'naked' or packaged (in complexes with lipids or polymers, and viruses) are delivered using magnetic nanoparticles (MNP) coated with cationic molecules. Through the use of magnetic nanoparticles complexed with gene vectors, therapeutic genes can be selectively targeted by an external magnetic field to tumor sites and other desirable target sites in order to increase the concentration of therapeutic genes while decreasing the exposure in the rest of the body. Since the technique involves administration of nano-sized particles containing magnetic particles and genetic material which are guided to the desirable site of action by an externally

applied magnetic field, it can be alternatively described as "MAGNET GUIDED NANOVECTORS FOR GENE DELIVERY".[19]

Some of the benefits of magnetofection are

- With high transfection efficiency with any nucleic acids it increases efficiency from 30 to 500%
- Powerful on hard-to-transfect and primary cells
- High performance even with low dose of nucleic acids (enable to use 10 to 100 times less nuclei acids)
- Concentrate genetic material onto cells / accelerate kinetics.[20]

Magnetofection is applicable to all types of nucleic acids (DNA, siRNA, dsRNA, shRNA, mRNA), non viral transfection systems (transfection reagents) and viruses. According to its defined applications, various optimized reagents have been designed in order to achieve good results.

The list of reagents commercially available for magnetofection have been tabulated along with their application in Table 2.[22]

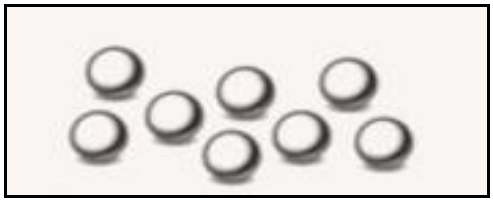
Table 2: List of reagents for magnetofection

REAGENT	COMBINATION AND APPLICATION
1. LipoMag Kit®	CombiMag + Dreamfect Gold reagents; for all nucleic acids
2. MagnetoFectamine®	CombiMag + Lipofectamine 2000; for all nucleic acids
3. PolyMag® / PolyMag Neo®	For all nucleic acids transfection
4. NeuroMag®	For neuron transfection
5. CombiMag®	For enhancing the efficiency of all transfection reagents
6. SilenceMag®	For siRNA applications
7. ViroMag®	For enhancing viral transduction efficiency
8. ViroMag R/L®	For Lentivirus and Retrovirus transduction
9. AdenoMag®	For Adenovirus and Adeno-associated virus transduction
10. Mag4C-LV® / Mag4C-AD®	For capturing and concentrating Lentiviruses and Adenoviruses respectively
11. FluoMag®	Fluorescent magnetofection reagent
12. SelfMag®	For creating your own magnetic delivery system

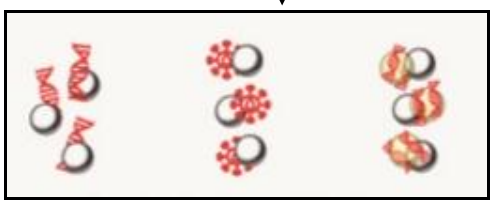
4.1. Protocol

The protocol for magnetofection is shown in Figure 3.[21]

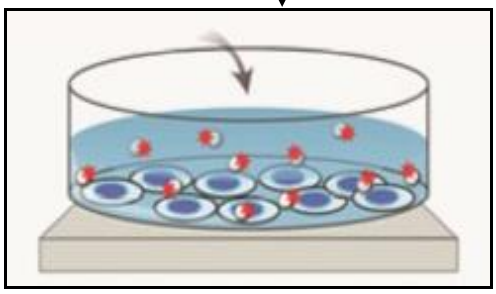
Nuclei Acids | Viral Particles | Lipoplexes Polyplexes



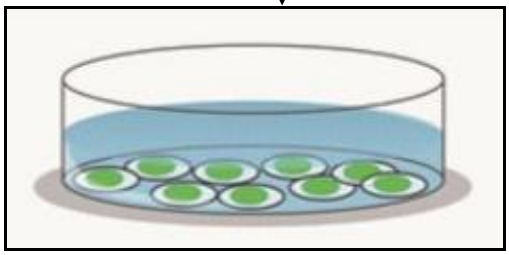
Magnetic Nanoparticles



Vectors complexed with MNPs



Magnetic complexes are added onto the cells



Cells are cultured

Step 1: Vector Preparation

a) Solutions of nucleic acids, viral particles or lipoplexes/ polyplexes are prepared in buffer or serum free culture medium.

b) MNPs are made of iron oxides (biodegradable) coated with specific proprietary cationic molecules.

Step 2: Magnetic Complex Formation

Vectors are mixed with magnetic nanoparticles formulation composing the Magnetofection™ reagents depending on the application and incubated for 20-30 minutes at room temperature.

Step 3: Magnetofection Procedure

Magnetic complexes are concentrated onto cells by the influence of an external magnetic field generated by a specific magnetic plate for 5-20 minutes. The cellular uptake of the genetic material is accomplished by two natural processes, endocytosis and pinocytosis.

Step 4: Culturing of cells

The magnetic force exerted upon the gene vectors allows a very rapid concentration of the entire applied vector dose onto cells/organs, so that all the target cells get in contact with a significant vector dose.

Figure 3: Protocol for Magnetofection

5. FACTORS AFFECTING EFFICIENCY OF MAGNETOFECTION

For successful transfection, there are several factors that need to be considered. They are enumerated as follows

a. Cell Type

As each cell type is likely to respond differently to a various transfection reagent or method, choosing the appropriate cell type for good results is important. Primary cultures mimic natural tissues very closely and are used more often. It is necessary to maintain a largely homogeneous population of cells while using primary cultures. However, they typically have a limited growth potential and life span, and are more difficult to maintain in culture. On the other hand, continuous cell lines are easier to work with in the laboratory, but they may not be suitable for modeling *in vivo* processes because of the multiple genetic changes that they have undergone.

b. Cell health and viability

The viability and general health of cells prior to transfection is an important source of variability from one transfection to another. Subculturing cells for at least 24 hours before transfection is recommended to ensure that they recover from the subculture procedure and are in optimum physiological condition for transfection. In general, cells should be at least 90% viable prior to transfection and should have had sufficient time to recover from passaging. Thawing a fresh vial of frozen cells and establishing low-passage cultures for transfection experiments allow the recovery of transfection activity. For optimal reproducibility, aliquots of cells of a low passage number can be stored frozen and thawed as needed. 3 or 4 passages after thawing a new vial of cells should be allowed. Since contamination can alter transfection results, cell cultures and media should be routinely tested for biological contamination.

c. Confluency

For optimal transfection results, following a routine subculturing procedure and passage cultures once or twice a week at a dilution that allows them to become nearly confluent before the next passage is important. Cells should not be allowed to remain confluent for more than 24 hours. For every new cell line to be transfected, the optimal cell density for transfection that varies with different cell types, applications, and transfection technology should be determined. Cells must not be confluent or in at the time of transfection, because actively dividing cells take up foreign nucleic acid better than quiescent cells. Too high of a

cell density can cause contact inhibition, resulting in poor uptake of nucleic acids and/or decreased expression of the transfected gene. However, too few cells in culture may result in poor growth without cell-to-cell contact.

d. Media

Different cell types have very specific medium, serum, and supplement requirements. Choosing the most suitable medium for the cell type and transfection method plays a very important role in transfection experiments. The information is usually available in published literature, or it can be obtained from the source of the cell banks. It is important to use fresh medium, especially for unstable components so that they don't harm cell growth.

e. Serum

The presence of serum in culture medium enhances transfection with DNA. Adding serum to media can boost cell growth but carries the potential of endotoxin contamination. For cationic lipid-mediated transfection, it is important to form DNA-lipid complexes in the absence of serum to avoid the interference of some serum proteins with complex formation.

While transfecting cells with RNA, transfection procedure must be carried out in the absence of serum to avoid possible contamination with RNAses. Most cells remain healthy for several hours in a serum-free medium. It is important to control for variability among different brands or even different lots of serum as the quality of serum can significantly affect cell growth and transfection result.

f. Antibiotics

In general, antibiotics can be present in the medium for transient transfection. Cationic lipid reagents can increase the amount of antibiotics delivered into the cells along with the increase cell permeability, resulting in cytotoxicity and lower transfection efficiency. Therefore, antibiotics should not be added to the transfection medium. When plating cells for transfection, antibiotics should be avoided as they can reduce the need for rinsing the cells before transfection. To create stable cell lines, 48 to 72 hours after the transfection procedure for cells should be allowed in order to express the resistance gene before adding the selective antibiotic. However, lower amounts of antibiotics can be used while using serum-free medium.[22]

g. Polymer selection: Polymers must endow MNPs with reactivity to nucleic acids which is based on physical interaction, i.e., electrostatic forces or on reactive residues through which

polymer and nucleic acid react. For the electrostatic conjugation of nucleic acids, polymers onto which DNA or RNA will be encapsulated must have a positive surface charge. Both polymer and nucleic acid must be modified with reactive chemical residues for covalent conjugation. Polymers used for gene delivery should protect nucleic acid from the acid microenvironment that frequently surrounds tumor tissues. Tumor cells must subsequently take up most of the nanocarriers that, once internalized, must evade lysosomal degradation to deliver the cargo into the cytosol.[23]

6. THERAPEUTIC INTERVENTION WITH MAGNETOFECTION

6.1. Magnetofection in cancer

Tumors grow quickly and stimulate the production of blood vessels. One of the side effects of the accelerated production is that the new vessels tend to be leaky which in turn allow particles to accumulate in the tumor, while the lack of adequate drainage means they remain there for longer than in healthy cells. This phenomenon is known as the "Enhanced Permeability and Retention Effect (EPR)" and is the one that nanotechnology exploits. Because of the extremely small size of MNPs, nanoparticles could slip through the leaky blood vessels and find cancerous cells anywhere in the body, even if they have metastasized or spread to other organs. Genetic material-loaded magnetic nanoparticles target the cancer cells and are endocytosed by cells under the guidance of an appropriate magnetic field. The applied magnetic field accelerates the nanoparticle transport.[15] The encapsulated material will be released into the cancer cells. The nanoparticles will also heat deep tumors that were previously inaccessible as the magnetic fields pass through the entire human body.[24]

Modified adenoviral vectors expressing LacZ conjugated with magnetic nanoparticles have been delivered effectively via direct injection into tumors of both the jejunum and the trachea. Beta-galactosidase activity was found to be significantly higher in the lung and jejunum in groups treated with magnetic nanoparticles, indicating enhanced cellular uptake, higher gene accumulation and retention and expression under an externally applied magnetic field.[25]

Fluorescently-labeled oligodesoxynucleotides were injected into femoral artery for targeting cremaster muscle and increased fluorescence was observed in the magnetic group. Enhanced fluorescence in the smaller arterioles confirmed that magnetic targeting enables better penetration of tumor tissue via its blood supply.[26] Magnetofection has been investigated as an in vivo cancer therapy using a human tumor xenograft mouse model. Magnetic

nanoparticle–lipid complex delivering a luciferase plasmid, was expressed significantly in experimental group of animals treated with both nanoparticles and an external magnet.[27] Similar result was observed when siRNA was directed against the EGF receptor in the magnetized groups, resulting in 50% reduction in tumor mass. New formulation consisting of smaller particles showed very little non-specific accumulation. Monocytes have recently been exploited as gene vectors for cancer therapy because of their endogenous origin as well as for their natural affinity towards tumors. Following ex vivo transfection of monocytes with marker genes, they are used to deliver therapeutic genes to tumors via the bloodstream.[28]

6.2. Magnetofection for gene delivery to internal organs

While magnetic gene delivery works best with external organs or tissues, internal organs have been effectively targeted using external magnets. It has been observed that reporter genes have been used in most studies investigating magnetic gene targeting of internal organs.

a) Liver

Gene therapy has shown promising results in treating hepatocellular carcinoma both in vitro and in vivo. These strategies include p53 gene replacement and RNAi mediated gene silencing and worked only when genes were directly applied to the liver. This allowed researchers to test the potential of gene therapy without a systemic delivery system. But, a systemic delivery system may be more broadly applicable for clinical applications due to the invasiveness of intratumoral injection.[29] Magnetically targeted gene delivery has been shown to significantly improve systemic delivery efficiency to internal organs. It has been reported that a group of scientists during transfection of a luciferase plasmid-magnetic liposome complex placed an external magnet near the liver and observed an increase in luciferase activity compared to a control group treated without an external magnet. Luciferase activity was decreased when a magnet was placed over the liver while analysing other internal organs, suggesting that magnetofection not only increases transfection at the target site, but also reduces exposure to other parts of the body.[30]

b) Kidney

Using similar conditions and techniques as for liver, kidneys have also been targeted with magnetic gene therapy. Scientists placed an external magnet between the hind legs of mice to target chitosan-based magnetic nanoplexes capable of expressing eGFP to the kidneys and observed much greater GFP fluorescence than a non-magnetized control. This study showed

that gene delivery to the kidney could be greatly enhanced by magnetic targeting. However, further studies on the effects on neighbouring tissue need to be performed.[31]

6.3. Magnetofection for gene delivery to the central nervous system

The blood brain barrier, an endothelial cell layer prevents therapeutic genes and many other drugs from entering the CNS. Delivery to the central nervous system (CNS) faces some challenge posed by these barriers. Two studies conducted on CNS-directed magnetic gene delivery have used direct injections to the CNS.

a) Spinal Cord

Magnetic nanoparticle/PEI complexes have been shown to be targetable following intrathecal injection in the spinal cord. Transfection at the injection site can be reduced due to circulation and diffusion of the cerebrospinal fluid. It has been reported that using a magnetic nanoplex to deliver a pCAG-luc plasmid, increased transfection at the injection site in the lumbar region was observed and on moving the magnet it could specifically target genes in the cervical region as measured by increased luciferase activity. For spinal tumors, this technique offers a unique method for targeting various regions of the spine by increasing the effect of a therapy at the tumor site and reducing exposure at other regions. Moreover, this approach potentially allows for the treatment of tumors in the cervical and thoracic regions of the spine by means of a lumbar puncture.[32]

b) Brain

Till now no studies have been performed on magnetic gene delivery to the developed brain. However, adenoviral vectors expressing GFP have been conjugated to magnetic nanoparticles and successfully targeted to a particular hemisphere of the rat embryonic brain. After direct injection into the 3rd ventrical of a rat embryo in uterus, scientists selectively delivered GFP to one side of the embryonic brain after application of an external magnet, as detected by fluorescence microscopy. This study showed promise for targeted delivery inside the brain. However, further studies need to be done in order to determine if this approach can be adapted for delivery of therapeutic genes to a fully developed adult brain.[33]

7. FUTURE PERSPECTIVE AND OTHER APPLICATIONS

Magentofection does not necessarily improve the overall performance of a given standard gene transfer method invitro, but its major advantage consists on the major possibility of remotely controlled vector targeting invivo.[19] However, the method of magnetofection

shares some of the challenges of gene therapy in general like poor expression of genetic material, risk of creating an immune response and toxicity or inflammation due to introduction of genetically altered viruses.

Despite some of the limitations of magnetofection, the technique can be exploited as an ideal research tool in the field of genetic engineering. It combines the benefits of particulate drug delivery as well as specific targeting of vectors.[34] It can be employed as a suitable tool for the study of gene function of developing organs and tissues. Gain-of-function and loss-of-function analysis was done following magnetofection of cDNA constructs and shRNA into mouse genital ridge tissue. It was studied by ectopic expression of Sry induced female-to-male sex-reversal and male-to-female sex-reversal induced by knockdown of Sox9 expression.[35] For in vitro application, the three important features of magnetofection include the drastically lowered vector dose; the considerably reduced incubation time required to achieve high transfection efficiency; and the possibility of gene delivery to otherwise non-permissive cells. These characteristics can make magnetofection particularly useful in the transfection/transduction of difficult-to transfect/transduce cells, for example in ex vivo gene therapy approaches.[34]

8. CONCLUSION

As gene therapy is uprising in the field of medicine, soon every genetic disease will have gene therapy as its treatment. The genetic testing, screening and research in finding the right gene is a daunting task. The short-lived nature of gene therapy makes the patients undergo multiple rounds of gene therapy and poses a s a great challenge.

Magnetofection acts as a reproducible method of gene delivery with auto matizable high throughput transfection efficiency and efficacy *in vitro* and in vivo. Various possibilities of the technique are being explored in recent times for treatment of cancers and any other disease where genetic aberration has been completely detected. Magnetofection provides a novel tool for high throughput gene screening in vitro and can help to overcome fundamental limitations to gene therapy in vivo.

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