Acta Scientific Pharmacology

Volume 2 Issue 4 April 2021

Review Article

# Gene Transfer Technologies and their Applications: Mitigation and Curing of Diseases

**Arghya Bhattacharya* and Lopamudra Chakravarty**

*Department of Pharmacology, Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences, West Bengal, India*

***Corresponding Author:** Arghya Bhattacharya, Department of Pharmacology, Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences, West Bengal, India.

**Received:** March 05, 2021

**Published:** March 30, 2021



## Abstract

Gene is considered as the segment of DNA which codes the RNA for polypeptide molecule. The efficient potential of structurally altered DNA molecule is focussing to generate new life-forms that are well adopted for surviving purpose. By Changing in the sequencing of base of DNA projects to change in structure of protein which produce disease condition and can be modified by manipulating the gene sequence. In previous years, vast techniques of transferring genes have evolved. Currently, with development of several unique strategies for gene therapy, successfully arranged gene delivery has found its importance again and become a major challenge in this selective field. The primary ways of gene transfer focussed in this article are electroporation, microinjection, macroinjection, biolistics or microprojectiles for DNA transfer, liposome mediated gene transfer, calcium phosphate mediated DNA transfer, DAE-Dextran for gene transfer, gene transfer by polycation-DMSO, polyethylene glycol mediated transfection and gene transfer through peptide. Much emphasis has been given to the gene manipulation in cardiovascular diseases, parkinson's disease, lysosomal disorders, ocular gene therapy, gene transfer in liver and osteoarthritis. This review article will let the reader to have a retrospective study on gene transfer technologies which manipulates the gene and also cure human diseases.

**Keywords:** Gene Transfer; Electroporation; Gene Gun; Macro Injection; Liposome; Biolistic; Retrovirus; Liver Therapy; Cancer Therapy

## Introduction

Gene therapy is the process of incorporating of genetic material into cells of the body in purpose of treatment of several human disease. Somatic cell gene therapy covers the genetic manipulation of different cell in body, only those involved in meiotic reproduction (germ line cells) are excluded, namely spermatozoa and ova. At present, over 200 clinical protocols worldwide that includes gene transfer into somatic cells, have been started or have received permission to start the procedure of conversely, germ-line gene therapy which has many scientific, moral and ethical issues and also are ready to be resolved and thus has not been as aggressively pursued to date.

The transfer of genetic material into a target cell provides a large window of potential applications in medicinal field. Inevitably, the demands about this are raised for clinicians as well as pathologists to have an understanding of its basic concepts, its therapeutic applications and its different pros and cons.

Throughout the past decades advancement has been made in the development of various techniques and reagents for the incorporation of macromolecules into eukaryotic cells. In this era, it is possible and has relevant way to incorporate genetic materials like DNA, RNA and proteins and small molecules into every types of cells. This review is actually based upon gene transfer systems.

Gene therapy includes therapeutic procedures in which recombinant gene(s) incorporate into human somatic cells and replace protein with a genetic defect or to interfere with pathological process of a disease [1-3]. When a gene signify a specific protein, is inserted into the nucleus of the specific target cell, it will be called as a template for mRNA synthesis which will ultimately help to activate the production of therapeutic protein which was in a patient's cell. This specific principle of gene transfer employed in practice by incorporating a gene within carrier molecules or vector and helps the DNA to be transported safely and efficiently into the nuclei of the target cells. The searching for an ideal vector for gene transfer is still in progress. Generally, there two main approaches which are followed:

a) Utilizing biological, viral vectors and b) utilizing either chemical or physical methods to incorporate the desired gene into target cells.

Firstly, viral gene transfer is the primary process in which a viral-mediated process is performed, referred as an infection. Secondly, non-viral gene transfer is the process for the treatment of cell by the help of chemical or physical means and the whole process is reffered as transfection.

The bio safety issues may also arise, based on the virus that is used. Alternatively in place of infection now a days researchers take the help of transfection for its safety issues and also it is faster and requires only a few reagents, plasmid DNA which is the main substance to carry the desired gene under the control of a strong cell-specific promotor. Transfection is classified into two major types including transient and stable. Transient transfection is the process which is temporary (i.e. expression of foreign gene that continues for long times) [4-8]. In contrast, stable transfection may occur with a lower frequency (10 to 100 – fold lower), but the expression is prolonged for the long term because the foreign DNA accumulates into the host genome.

This review is rather focused on three areas. Firstly the description of several vectors of specific importance on the safety issues and the pros and cons of these systems. The second will focus on gene transfer into hematopoietic cells, which, largely because of their ease of access, have been the most widely investigated cells in gene transfer experiments. The effects of gene marking studies and potential applications for gene therapy by using hematopoietic cells, will also be considered. The last section will help to explore the potential of gene transfer for cancer therapy.

## Historical perspective

In 1928, Frederick Griffiths discovered a transforming agent in a virulent strain of Pneumococcus which was able to include virulence factor into non-virulent strain. These discoveries gives the hint at the potential for using gene transfer as a therapeutic tool. The origin of recombinant DNA technology 25 years ago provided tools which would be essential for effective gene therapy including cloned genes and feasible gene transfer techniques [6-12]. Despite substantial progress having been made since the first gene transduction into mammalian cells using the calcium phosphate method, the search for the perfect vehicle to carry genes into target cells has remained elusive.

## Several technologies for gene transfer

### Electroporation

Electroporation utilizes electrical pulse to produce transient pores in the plasma membrane and allow macromolecules into the cells. It is an active process to transfer DNA into cells. Microscopic pores are incorporated in biological membrane by the help of electric field. These pores are called electropores which permit the molecules, ions and water to cross from one side of the membrane to another. The pores can be recovered only by applying a suitable electric pulse. The formation of electropores based upon the cells that are used and the amplitude and duration of the electric pulse that is applied to them.

In case of mammalian transgenesis, electroporation is an active method of introducing exogenous DNA into embryonic stem (ES) cells [2]. This technique has recently, been used to transfer genes into cultured mammalian embryos at selective stages of development [3,4]. It was reported [5] that there is an increase from 12 to 19% of transgenic bovine blastocysts when electroporation was included in an otherwise passive sperm-DNA uptake protocol. Similar findings were reported with transgenic bovine blastocysts. Fish species were also evidenced to be genetically manipulated in this possible way [7,8].

**Figure 1:** Electroporation method of gene transfer.

### Microinjection

In microinjection DNA can be introduced into cells or protoplast with the help of very fine needles or glass micropipettes having the diameter of 0.5 to 10 µm. Few DNA injected may be abstracted by nucleus. Computerized control of holding pipette, needle, microscope stage and video technology has improved the efficacy of this technique. Microinjection is potentially a effective technique for simultaneous introduction of multiple bioactive compounds such as antibodies, peptides, RNAs, plasmids, diffusion markers, elicitors, Ca2+ as well as nucleus and artificial micro or nanoparticles consisting those chemicals into the corresponding single-cells target.

### Macroinjection

Macroinjection is the method used for transferring artificial DNA to cereals plants that show inability to regenerate and develop into whole plants from cultured cells. Needles used for injecting DNA are with the diameter greater than cell original diameter. DNA injected with traditional syringe into region of plant which will help to develop into floral tillers. All over in 0.3 ml of DNA solution is injected at a point above tiller node until several drops of solution appears from top of young inflorescence [15-18]. Timing of injection is primary and should be fourteen days before meiosis. This method was identical with rye plants. It is also being attempted for other cereals plants also.

### Biolistics or microprojectiles for DNA transfer

Biolistics or particle bombardment is a physical method that uses accelerated microprojectiles for delivering DNA or other molecules into intact tissues and cells [20]. The method was developed initially to transfer genes into plants [21,22]. Biolistics transformation is the newest method among the physical methods for transferring exogenous DNA artificially. This method excludes the need of protoplast and is better in efficacy. This technique can be utilized for any plant cells, root section, embryos, seeds and pollen. The gene gun is a device that makes firing of DNA into selective target cells [23,24]. The DNA to be transformed into the cells is coated onto microscopic beads and consist of either gold or tungsten. Beads are carefully coated with DNA. The beads that are coated are binded to the end of the plastic bullet and packed into the firing chamber of the gene gun. An explosive force that fires the bullet down the barrel of the gun towards the target cells that ultimately lie just beyond the end of the barrel. When the bullet outstretched at the end of the barrel it is caught and stopped, but the DNA coated beads continue on toward the selective target cells. Some of the beads crossed through the cell wall into the cytoplasm of the target cells. Here the bead and the DNA dissociate and the cells become modified. Once it reached inside the target cells, the DNA is solubilised and may be expressed [25].

### Bead transduction

This method of transduction combines the principle of physically producing breaks in the cellular membrane, as utilised in the scrape loading technique with the use of beads, as described for the loading of macromolecules. The efficiency, although relatively low, is equivalent to Electroporation.

### Gene gun or particle bombardment

The gene gun applies heavy metal particles (either tungsten or gold, 1-5.um in diameter) that are stimulated and fired to a high velocity which is sufficient to penetrate the target cells, and it persists to be a vital technique of plant cell transduction. Recently this technology has been applied to animal cells. These devices use a helium-driven plunger to pierce a Kapton disc placed in front of another disc on which the DNA-coated microprojectiles are fixed. When the gas is released, a shockwave three to four times the speed of sound is created and this launches micro projectiles against the target tissue. Again the efficiency is low and the DNA is not incorporated into the genome 33.

### Liposomes

This method utilises lipid chemistry to surround naked DNA plasmids with a liposomal coat which is subsequently endocytosed by the target cell. Plasmid liposome complexes have many advantages as gene transfer vectors: they can be used to transfer expression cassettes of essentially unlimited size, cannot replicate or recombine to form an infectious agent and may evoke fewer in-

flammatory or immune responses because they lack proteins. The optimum disadvantage of these vectors is that they are inefficient, need thousands of plasmids should be presented to the target cell to achieve successful gene transfer.

**Figure 2:** Liposome mediated method of gene transfer.

### Calcium phosphate mediated DNA transfer

The process of transfection involves the admixture of isolated DNA (10-100ug) with solution of calcium chloride and potassium phosphate under condition which allow the precipitation of calcium phosphate to be formed. Incubation of cells are then made with precipitated DNA either in solution form or in tissue culture dish. A fraction of cells will consume the calcium phosphate DNA precipitate by endocytosis process. Transfection efficiencies using calcium phosphate can be quite low, in the range of 1-2% [38]. Procedures have been modified where cells are taking up exogenous DNA could be up to 20%.

### DNA transfer by DAE-Dextran method

DNA can be transferred with the application of DAE Dextran. DAE-Dextran may be utilized in the transfection medium in which DNA is present. This is polycationic, high molecular weight substance and suitable for transient assays in cos cells. It does not seems to be efficient for the production of stable transfectants. If DEAE-Dextran treatment is coupled with Dimethyl Sulphoxide (DMSO) shock, then up to 80% transformed cell can express the transferred gene. It is evidenced that serum halts this transfection so cells are washed nicely to make it free of serum [36]. Stable expression is very hard to obtain by this procedure. Treatment with chloroquinine increases transient expression of DNA. The primary advantage of this method is that, it is cheap, simple and can be utilized for transient cells which may not be able to survive even by short exposure of calcium phosphate.

### Transfer of DNA by polycation-DMSO

Calcium phosphate method of DNA transfer is consistent and appropriate but there is narrow range of identical conditions. DNA transfer by polycation, polybrene is used to increase the adsorption of DNA to the cell surface followed by a brief treatment with 25-30% DMSO to increase membrane permeability and enhance uptake of DNA. In this method carrier DNA is absent and stable transformants are produced. This method produce works with mice fibroblast and chick embryo [28].

### Polyethylene glycol mediated transfection

This method is utilized for protoplast only. Polyethylene glycol act by stimulating the process of endocytosis and therefore DNA uptake takes place. Protoplasts are preserved in the solution consisting of polyethylene glycol (PEG). The molecular weight of PEG utilized is 8000 dalton and having the final concentration of 15%. Calcium chloride is mixed and sucrose and glucose plays a key role as osmotic buffering agent. For reducing the effects of nuclease present, the carrier DNA from salmon or herring sperm may also be included. After getting the exposure of the protoplast to exogenous DNA in presence of PEG and other chemicals, PEG is permitted to get removed. Surviving protoplasts are then final cultured to form cells with walls and colonies. PEG based vehicles were less toxic and more resistant to nonspecific protein adsorption making them an attractive alternative for nonviral gene delivery [31]. PEG PBLG nanoparticle mediated HSV TK/GCV gene therapy process for oral squamous cell carcinoma was also evidenced [33].

### Gene transfer through peptide

A variety of peptide sequences are there which are able to bind to, and condense, DNA to make it more amenable for entry into cells. The tetrapeptide known as “Serine - proline-lysine-lysine” that present on the C- terminus of the histone and plays a key role in DNA transfer [40]. Lysine is a positively charged amino acid. The lysine which is positively charged amino acid, its side chains help to counteract with the negatively charged phosphate DNA backbone and utilize the DNA molecules to pack closely to each other. Rational design of peptide sequences has also been utilized to produce synthetic DNA binding peptide. Tyrosine-lysine-alanine-(Lysine)

s-tryptophan-lysine is a peptide which is very identical to produce complexes with DNA [37]. DNA binding peptides which can be able to couple with cell specific ligands also be synthesized. This process allows the receptor regulated targeting of the peptide/DNA complexes to selective cell types.

### Gene transfer by retroviruses

The relatively low potency of foreign DNA accumulation into animal cells, incorporated with the deficiency of naturally occurring plasmids, projecting to the manipulation of viruses as potential vectors for gene transfer. The genome of retroviruses can be altered to convey exogenous DNA. The unification of a single copy of the viral DNA at a random location within the host's genome allows for the long term expression of the integrated foreign gene. Virus can be utilized for highly matured tissues, like those of foetuses, juveniles or adults [26-28]. This gives great success in the field of somatic gene therapy. Retroviral vectors have also been utilized to incorporate transgenes into the ES cell genome [24-30]. Still, retroviral vectors has several limitations in a number of respects. They show random nature of integration process, which may sometimes have adverse effects on the host cell, and the general requirement that retrovirus have to attack only those cells that are dividing continuously.

### Applications to human diseases

Gene therapy is a key factor that utilizes effective gene delivery into live cells. Gene therapy is selectively demanding approach for diseases which currently do not have any fruitful treatment options, and is probably easier for monogenic disorders than complex diseases. Gene transfer provides its application in wide range of diseases including cardiovascular disease, parkinson's disease, lysosomal disorders, ocular gene therapy, liver disorder, osteoarthritis and a number of other diseases.

### Cardiovascular disease

Cardiovascular disease (CVD) is still be a reason of death in industrialized countries, though the substantial development made during the last few years in the mitigation and treatment of cardiovascular events. Application of gene therapy in the era of cardiovascular disorders has been the become the subject of intensive work over the current period. Recent human clinical trials have evidenced that injection of naked DNA encoding vascular endothelial growth factor advances collateral vessel development in patients with critical limb ischemia or chronic myocardial ischemia. Several studies in animals have also fuelled enthusiasm for treatment of human restenosis by gene therapy, but clinical applications are warranted. Application of gene transfer to other cardiovascular diseases must require the coordinated improvement of a variety of recent technologies, as well as a better definition of cellular and gene targets [36-38].

### Parkinson's disease

The risk/benefit ratio for these techniques is now generally thought to be acceptable under approved protocols. The currently used vehicle for gene delivery to the brain of human is known as recombinant adeno-associated viral vector, which is nonpathogenic and non–self-amplifying [16-20]. Candidate genes tested in PD patients encode glutamic acid decarboxylase, which is injected into the subthalamic nucleus to catalyze biosynthesis of the -aminobutyric acid and so essentially mimic deep brain stimulation ofγinhibitory neuro transmitter this nucleus.

### Lysosomal disorders

They are occurred due to the insufficiency of specific lysosomal enzymes, hydrolases involved in the catabolism of macromolecules (glycolipids, glycoproteins, glycosaminoglycans, etc.), projecting to intracellular aggregation of storage material. Diseases related to lysosomal storage are monogenic metabolic disorders which results from insufficiency in intra lysosomal enzymes used in macromolecule catabolism. Different groups have been described according to the affected pathway and the integrated substrate: muco polysaccharidoses, lipidoses, glycoproteinoses and glycogenosis type II. Their clinical severity and the insufficient therapy for the majority of these disorders justify the development of gene transfer methods [22-28]. Maximum genes encoding the normal lysosomal enzymes have been replicated and currently several animal models have been evidenced for nearly all these marked diseases.

### Recent developments in ocular gene therapy

The eye exhibits unique features for the development of successful gene therapy. An additional opportunity of utilizing the eye for gene therapy is the possibility of assessing the development of the treatment in a noninvasive technique by directly measuring visual function or activity. Delivery of exogenous genes to the eye in vivo is capable of treating several ocular diseases by using the gene as drugs. Genes have many additional advantages over convention-

al drugs. When the gene moves inside the cell they are able to show their products for prolonged periods of time that greatly exceed the duration of action of recently available drugs. Most of the eye diseases are chronic and progressive including macular degeneration and glaucoma, or that some are produced by an increased number of mutations including retinal degeneration, this facet of gene therapy holds a vital role [28-32]. No inflammation is still at six days after the expression of LacZ gene. In another study, naked plasmid DNA was injected into the cornea of mice and continued for 10 days [22]. Photoreceptors may be defined as the retinal neurons which are specialized in converting the light entering the eye into a neural signal. Retinitis pigmentosa (RP) is an example. There is no cure or successful treatment. Several attempts to halt the death of photoreceptors by gene-mediated therapy have been raising successfully [32-36].

### Gene transfer in liver

Hepatic gene therapy provides us better approach for the mitigation and cure of metabolic defects or serum protein insufficiencies. Treatment of genetic diseases by applying therapeutic gene delivery gives a number of complications, including the need for a stable, therapeutic expression of the transferred genes. Gene delivery by applying retroviruses projects in long term expression because they accumulate into cell chromosomes. In addition with, utilization of retroviruses for in vivo gene transfer has been halted by the need for cell proliferation as retroviruses only accumulate into dividing cells [37]. Follistatin administered intraportally to 50% hepatectomized rats accelerated liver regeneration, increased the number of hepatocytes cycling, and was quite effective in promoting gene delivery with retroviral vectors [38].

### Osteoarthritis

Osteoarthritis is a bone disease that affects over 43 million Americans, a number that is predicted to rise to 60 million by the year 2020. This disease is not only incurable also the treatment is insufficient, but it is also very exhausting, projecting to physical impairment, hampered in quality of life and working days also lost [38-40]. Gene transfer to the synovial linings of affected joints is a well known remedial approach for obtaining supportive, therapeutic, and intra articular concentrations of antiarthritic gene products. Several non-viral vectors have been tested for their capability to transfect the synovia of experimental animals following intraarticular injection. None have them provides more than lower levels of short term transgene expression and are also inflammatory. Lots of viral vectors are very effective in this field and fruitfully treats several experimental models of osteoarthritis.

## Conclusion

Thus there are vast ways by which the gene can be incorporated inside the cells. By the application of molecular tools and technologies it becomes easy and safe to incorporate the gene into cells without damaging its integrity and biological function. Additionally the current development in molecular biology has made possible the transfer of gene with more accuracy to the selective cell. The transfer of gene technologies has provided of way of curing from a number of diseases. The treatment of diseases by gene transfer process gives better output for a prolong period of time. Now it is just the need of patience and time to develop new, cheap and safer process of gene transfer technologies to make the treatment of the diseases a little bit easier and affordable by all.

## Bibliography

1. Orkin SH and Motulsky AG. "NIH Proceedings-Dec 95 Report and recommendations of the panel to assess the NIH investment in research on gene therapy" (1995).

2. Wivel NA and Walters L. "Germ-line gene modification and disease prevention: some medical and ethical perspectives". *Science* 262 (1993): 533-537.

3. Raven PH and Iohnson JB. "Biology" 2nd editionn. St Louis: Times Mirrorf Mosby College Publishing, 1989 The genetic material (1989).

4. Avery OT., *et al*. "Studies on the chemical nature of the substance inducing transformation of pneumococcal types". *Journal of Experimental Medicine* 79 (1944): 137-158.

5. Watson ID., *et al*. "Molecular Biology of the Gene" 4th ed. The Benjamin/Cummings Publishing Company Inc, The extraordinary diversity of eukaryotic viruses (1988).

6. Temin HM. "The Retroviradae". New York: Plenum Press, Origin and general nature of retroviruses (1992).

7. Coffin IM. "Virology". New York: Raven Press Ltd. Retroviradae and their replication (1992).

8. Kavanaugh MP., *et al*. "Cell-surface receptors for gibbon ape leukemia virus and amphotropic murine retrovirus are inducible sodium-dependent phosphate symporters". *Proceedings of the National Academy of Sciences of the United States of America* 91 (1944): 7071-7075.

9. Van Zeul M., *et al*. "A human amphotrophic retrovirus receptor is a second member of the gibbon ape leukemia virus receptor family". *Proceedings of the National Academy of Sciences of the United States of America* 91 (1994): 1168-1172.

10. Miller DG., *et al*. "Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection". *Molecular and Cellular Biology* (1990): 4239-4242.

11. Porter CD., *et al*. "Comparison of efficiency of infection of human gene therapy target cells via four different retroviral receptors". *Human Gene Therapy* 7 (1996): 913-919.

12. Gelinas C and Temin HM. "Nondefective spleen necrosis virus-derived vectors define the upper size limit for packaging reticuloendotheliosis viruses". *Proceedings of the National Academy of Sciences of the United States of America* 83 (1986): 9211-9215.

13. Donahue RE., *et al*. "Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer". *Journal of Experimental Medicine* 176 (1992): 1125-35.

14. Trapnell BC and Gorziglia M. "Gene therapy using adenoviral vectors". *Current Opinion on Biotechnology* 5 (1994): 617-25.

15. Kremer El and Perricaudet M. "Adenovirus and adeno-associated virus mediated gene transfcr". *British Medical Bulletin* 51 (1995): 31-44.

16. Ginsberg HS. "The Adenoviruses". New York: Plenum (1984).

17. Chroboczek J., *et al*. "The sequence of the genome of adenovirus type 5 and its comparison with the genome of adenovirus type 2". *Virology* 186 (1992): 280-285.

18. Graham PL., *et al*. "Characteristics of a human cell line transformed by DNA from human adenovirus type 5". *Journal of General Virology* 36 (1977): 59-72.

19. Bett AI., *et al*. "Packaging capacity and stability of human adenovirus type 5 vectors". *Journal of Virology* 67 (1993): 5911-5921.

20. Bett AJ., *et al*. "An efficient and flexible system for construction of adenovirus vectors with insertions and deletions in early regions 1 and 3". *Proceedings of the National Academy of Sciences of the United States of America* 91 (1994): 8802-8806.

21. Parks RI., *et al*. "A helper-dependent adenovirus vector system: removal of the helper virus by Cre-mediated excision of the viral packaging signal". *Proceedings of the National Academy of Sciences of the United States of America* 93 (1996): 13565-13570.

22. Michael SI., *et al*. "Strategies to accomplish targeted gene delivery implying modified recombinant adenoviral vectors". *Cancer Gene Therapy* (1995): 321.

23. Wickham TJ., *et al*. "Targeted adenovirus-mediated gene delivery to T cells via CD3". *Journal of Virology* 71 (1997): 7663-7669.

24. Wickham TJ., *et al*. "Targeting of adenovirus penton base to new receptors through replacement of its RGD motif with other receptor-specific peptide motifs". *Gene Therapy* 2 (1995): 750-756.

25. Neve RL and GeHer AJ. "A defective herpes simplex virus vector system for gene delivery into the brain: compatison with alternative gene delivery systems and usefulness for gene therapy". *Clinical Neuroscience* 3 (1996): 262-267.

26. Turner SL and Jenkins FJ. "The roles of herpes simplex virus in neuroscience". *Journal of Neurovirology* 3 (1997): 110-125.

27. Latchman DS. "Herpes simplex virus vector for gene therapy". *Molecular Biotechnology* 2 (1994): 179-95.

28. Toneguzzo F and Keating A. "Stable expression of selectable genes introduced into human hematopoietic stem cells by electric field mediated DNA transfer". *Proceedings of the National Academy of Sciences of the United States of America* 83 (1986): 3496-3499.

29. Wu D and Keating A. "Engraftment of donor-derived bone marrow stromal cells". *Experimental Hematology* 19 (1991): 485.

30. Fechheimer M., *et al*. "Transfection of mammalian cells with plasmid DNA by scrape loading and sonicating loading". *Proceedings of the National Academy of Sciences of the United States of America* 84 (1987): 8463-8467.

31. MacNeil PL and Watder E. "Glass beads load macromolecules into living cells". *Journal of Cell Science* 88 (1987): 669-678.

32. Mathews KE., *et al*. "Bead transfection: rapid and efficient gene transfer into marrow stromal and other adherent mammalian cells". *Experimental Hematology* 21 (1993): 697-702.

33. Mathews KE and Keating A. "Gene therapy with physical methods of gene transfer". *Transfusion Science* 17 (1996): 29-34.

34. Ratajczak MZ and Gewirtz AM. "The biology of hematopoietic stem cells". *Seminar on Oncology* 22 (1995): 210-217.

35. Ogawa M. "Differentiation and proliferation of hematopoietic stem cells". *Blood* 81 (1993): 2844-2853.

36. Orlic D and Bodine DM. "What defines a pluripotent hematopoietic stem cell (PHSC): will the real PHSC please stand up!" *Blood* 84 (1994): 3991-3994.

37. Salmons B and Gupta C. "Targeting retroviral vectors for gene therapy". *Human Gene Therapy* 4 (1993): 129-141.

38. Carter RF., *et al*. "Autologous transplantation of canine long-term matrow culture cells genetically marked by retroviral vectors". *Blood* 79 (1992): 356-364.

39. Sekhat M., *et al*. "Retroviral transduction of CD34-enriched hematopoietic progenitor cells under serum-free conditions". *Human Gene Therapy* 7 (1996): 33-38.

40. Hatzfeld A., *et al*. "Increased stable retroviral gene transfer in early hematopoietic progenitors released from quiescence". *Human Gene Therapy* 7 (1996): 207-213.