Aus der
Klinik und Poliklinik für Herzchirurgie am Universitätklinikum Rostock
Direktor: Prof. Dr. med. habil. Gustav Steinhoff

Polymervermittelter Gentransfer für die Therapie mit adulten Stammzellen

Dissertation

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Abbreviations

ASO	Antisense oligonucleotide
BMP	Bone morphogenetic protein
B-PEI	Branched-polyethylenimine
cm	Centimeter
CO_2	Carbon dioxide
CTAB	Cetyltrimethylammonium bromid
Da	Dalton
DNA	Deoxyribonucleic acid
DOGS	Dioctadecylamidoglycylspermine
DOPC	1,2-dioleoyl-sn-glycero-3-phosphatidylcholine
DOPE	1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine ,
DOTAP	N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate
DOTMA	N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride
DMRIE	1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide
ECM	Extracellular matrix
EF	Ejection fraction
G1-phase	Gap 1-phase
G2-phase	Gap 2-phase
GAC	Gene activated collagen
GAH	Gene activated human fibronectin
GAS	Gene activated substrate
GFP	Green Fluorescent Protein
HIV	Human immunodeficiency virus
HPF	High-power field
HSC	Hematopoietic stem cell
kb	Kilobase pairs
KGF-1	Keratinocyte growth factor-1
LMW-PEI	Low molecular weight-polyethylenimine
LNA	Locked nucleic acid
L-PEI	Linear-polyethylenimine
LV	Left ventricular

LWT	Left ventricle wall thickness
mg	Milligram
MHz	Megahertz
MI	Myocardial infarction
MNB	Magnetic nanobead
MMP-2	Matrix metalloproteinase-2
M-phase	Mitosis-phase
mRNA	Messenger RNA
MSC	Mesenchymal stem cell
NLS	Nuclear localization signal
nm	Nanometer
NPC	Nuclear pore complexes
N/P ratio	Nitrogen/phosphorus ratio
PAMAM	Polyamidoamine
PBS	Phosphate buffered saline
PEI	Polyethylenimine
PLL	Poly-L-lysine
PPE	Polyphosphoester
RLU	Relative light unit
RNA	Ribonucleic acid
sc	Scrambled control
SDF-1α	Stromal cell-derived factor-1α
siRNA	Small interfering ribonucleic acid
S-phase	Synthesis-phase
SV40	Simian vacuolating virus 40 *or* Simian virus 40
VEGF	Vascular endothelial growth factor
W	Watt
µg	Microgram
µm	Micron

Zusammenfassung

Gentransfer, die Technik, mit der Genmaterial in Zielzellen bzw. –gewebe eingebracht wird, hat über die letzten Dekaden starkes Interesse hervorgerufen. Aus therapeutischer Sicht handelt es sich dabei um einen vielversprechenden Ansatz zur Behandlung verschiedenster, sowohl erblicher als auch erworbener Erkrankungen. In der wissenschaftlichen Laborarbeit wird Gentransfer als unersetzliches experimentelles Werkzeug bei der Erforschung von Genfunktionen angewandt. Der virale Gentransfer hat den Vorteil einer hohen Transduktionseffizienz, geht aber mit diversen Nachteilen einher: Toxizität, Immunogenität, Kanzerogenität, niedrige Zielzellspezifität, begrenzte Größe transfizierbarer Gene sowie hohe Kosten. Infolgedessen hat der nonvirale Gentransfer steigende Beachtung gefunden, da er relativ sicher ist, den Transfer großer Gene sowie ein spezifisches Targetting ermöglicht, weniger Toxizität und geringere Kosten verursacht. Von verschiedenen Verfahren zum nonviralen Gentransfers wurde vor allem der Polyethylenimin (PEI)-vermittelte Gentransfer intensiv erforscht und eingesetzt, da PEI sowohl *in vitro* als auch *in vivo* hervorragend wirkt.

Adulte Stammzellen sind undifferenzierte Zellen, die zur Selbsterneuerung fähig sowie multipotent sind. Aufgrund ihrer Fähigkeit, zu verschiedenen Zelltypen auszudifferenzieren, spielen Stammzellen in der Regenerativen Medizin eine wesentliche Rolle. Allerdings begrenzen einige Einschränkungen ihre therapeutische Wirksamkeit. Beispielsweise könnten Zellalterung und alterungsbedingter Funktionsabbau den Nutzen einer klinischen Stammzelltransplantation verringern. Durch die begrenzte Menge gewebeständiger Stammzellen stehen zudem nicht immer genug Zellen zur Verfügung, um geschädigtes Gewebe zu reparieren und zu regenerieren. Mit Hilfe des Gentransfers könnte diese Einschränkungen entgegengewirkt werden.

In der vorliegenden Arbeit wurden Methoden des polymervermittelten, nonviralen Gentransfers untersucht und in der Stammzelltherapie eingesetzt. Wir haben ein genaktiviertes Substrat (GAS) entwickelt, das eine lokalisierte Genapplikation sowie eine langanhaltende Genfreisetzung bei hoher Transfektionseffizienz und niedriger Zytotoxizität ermöglicht. Das GAS könnte für gezielte Stammzellmigration und –homing sowohl *in vitro* als auch *in vivo* eingesetzt werden und bietet so die Möglichkeit, Einschränkungen durch die geringe Anzahl Stammzellen in gewebeständigen Populationen zu überwinden. In einer unserer Studien haben wir gezeigt, dass die

Stammzellrekrutierung die Wiederherstellung der Herzfunktion nach Myokardinfarkt (MI) im Rattenmodell verbessern konnte. Dieses Ergebnis stellt eine weitere Bestätigung des therapeutischen Potenzials von GAS für die Geweberegeneration dar. Außerdem haben wir zum besseren Verständnis der Genmodifikation von Stammzellen die Transfektion humaner mesenchymaler Stammzellen (MSCs) durch PEI-vermittelten Gentransfer untersucht. Wir konnten zeigen, dass die Effizienz des Gentransfers unabhängig von Alter und Geschlecht des Stammzellspenders war, aber eine Abhängigkeit vom Zellzyklus aufwies. Als wesentliches Ergebnis zeigte sich, dass die Expression therapierelevanter Gene durch PEI signifikant verstärkt werden könnte, bis ein klinisch bedeutsames Niveau erreicht wird. Damit bietet der PEI-vermittelte Gentransfer die Möglichkeit, Stammzellen genetisch zu verändern und so ihre therapeutische Wirksamkeit zu verbessern. Außerdem konnten wir in einer weiteren Studie mittels einer nonviralen Methode Antisense-Oligonukleotide (ASO) verabreichen, so dass eine erfolgreiche Hemmung des Wachstums von Tumoren beobachtet wurde.

Summary

Gene delivery, the technique to introduce genetic materials into hosts, has drawn a lot of attentions in the last decades. On bed side, it is a highly promising therapeutic approach to treat various diseases, either inherited or acquired disorders. On bench side, it is an invaluable experimental tool to study gene functions. Viral gene delivery owns the advantage of high transduction efficiency, but it may be associated with drawbacks including toxicity, immunogenicity, carcinogenicity, poor target cell specificity, inability to transfer large size genes and high costs. As a result, non-viral gene delivery has attracted increasing interest since it presents relative safety, ability to transfer large size gene, less toxicity, site-specificity and low cost. Among various methods of non-viral gene delivery, polyethylenimine (PEI) mediated gene transfer has been widely studied and utilized due to PEI's excellent performance both *in vitro* and *in vivo*.

Adult stem cells are undifferentiated cells holding the properties of self-renewal and multipotency. Owning to their capability to differentiate into various cell types, stem cells have been playing an important role in regenerative medicine. However, some restrictions limited the therapeutic efficacy of stem cells. For example, cellular senescence and age-related functional decline could reduce the benefits after their clinical transplantation. Others include the limited tissue intrinsic stem cell pools, which can not provide enough stem cells to repair and regenerate damaged tissues. These limitations imposed on stem cell-based therapy could be addressed by gene transfer approach.

In present work, polymer mediated non-viral gene delivery technique was studied and utilized in stem cell-based therapy. We developed the gene activated substrate (GAS) which allows localized gene delivery, sustained gene release, high transfection efficiency and low cytotoxicity. This GAS could be used to guide stem cell migration and homing both *in vitro* and *in vivo*, providing the possibility to overcome the limitation of low stem cell amount in intrinsic tissue pools. In one of our studies, we have demonstrated that stem cell recruitment could improve the restoration of heart functions after myocardial infarction (MI) in a rat model. This result further confirmed the therapeutic potential of GAS in tissue regeneration. In addition, to improve our understanding in genetic modification of stem cells, we studied the transfection of human mesenchymal stem cells (MSCs) using PEI mediated gene delivery. We found

that the gene transfer efficiency is independent on the donors' age and gender, but shows relationship with the cell cycle. Importantly, the therapeutic gene expression level could be significantly enhanced by PEI to a clinical meaningful level. Hence, PEI mediated gene delivery offers the opportunity to genetically modify stem cells and thereby to improve their therapeutic efficacy. Furthermore, in another study, by using non-viral method to deliver antisense oligonucleotide (ASO), the successful inhibition of tumor growth was observed.

Introduction

1. Gene therapy

"Gene therapy" is a broad term that comprises any strategy to treat a disease by transferring nucleic acid materials into cells thereby regulating cellular processes and responses [1, 2]. Although the concept of gene therapy originally refers to the transfer of DNA, it currently includes the transfer of other nucleic acids materials like RNA [3], oligonucleotides [4] or single-stranded pieces [5].

Compared with traditional protein therapy, in which therapeutic proteins are given directly to the cells, gene therapy owns some advantages due to its capability to conquer the inherent problems of protein therapy such as systemic toxicity, *in vivo* clearance and high costs. The original aim of gene therapy was to treat some inherited genetic disorders such as cystic fibrosis [6]. Nowadays, it has been used for numerous disease treatment, including HIV [7], cancer [8], tissue regeneration [9] and diabetes [10] etc.

In gene therapy, the alternation or manipulation of genes or gene expression within a specific cell population of the host could be realized through the transfer of exogenous genetic materials, which provides the opportunities not only for clinical application but also for mechanism studies. Gene-related immunization can be acquired via appropriate gene transfer [11]. Gene transfer technique has become a powerful tool for researchers to identify the gene function and its regulation, thereby to establish various DNA-based disease models, and finally to explore potential therapeutic methods to various diseases, either inherited or acquired. Recently, the highly developed techniques in molecular biology combined with the culmination of the "Human Genome Project" have speeded up the understanding on cellular processes and disease pathogenesis [12, 13]. Numerous genes involved in diseases and cellular processes have been identified and the identification rate of those unclear target genes are dramatically increased with the usage of new techniques. All of these create promising prospects for gene therapy.

1.1 Gene therapy strategies

Generally, there are two main strategies adopted in gene therapy, *ex vivo* and *in vivo* gene transfer (Figure 1) [14]. *Ex vivo* gene therapy is carried out by transfer genes

into the cells of interest that are previously obtained from the tissue or organs of the patients. The cells are cultured *in vitro* in appropriate culture conditions, and then transfected (or transduced) with the certain therapeutic genes. After transfection (or transduction), the cells will be transplanted back into the patient. To get enhanced therapeutic efficacy, the positively transfected cells can be selected out from the total cells for transplantation according to their ability to express the exogenous gene in a stable and persistent manner. In some cases, allogenic cells or even allogenic cell lines could be used instead of autologous cells if the organ or tissue of interest is difficult to extract or it is hard to culture *in vitro* [15]. In *in vivo* gene transfer, the therapeutic genes are delivered directly into the tissue or organs [16, 17]. The gene transfer can be performed either via systemic injection in which the genes are intravenously injected into blood stream, or via *in situ* injection in which the genes are injected into tissue or organs of interest. The successful treatment critically depends on the gene transfer efficiency and the expression efficiency of the gene.

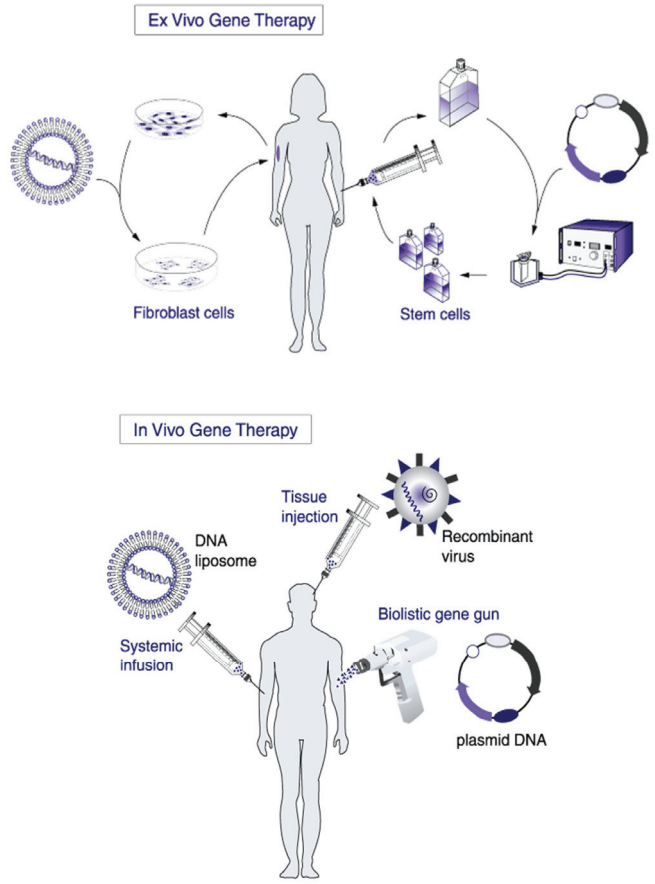

Figure1. *Ex vivo* and *in vivo* gene therapy strategies
(Pictures are from http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture25/Lecture25.html)

In theory, the successfully introduced therapeutic genes have several functions in accordance with the treating purpose and the gene transfer methods (Figure 2): 1) through gene modification, the defective host gene may be partially restored by directed mutagenesis; 2) gene replacement may exchange the defective host gene with the therapeutic gene which is the normal version; 3) through gene insertion, a therapeutic gene may be inserted into the host genes to exert the therapeutic action; 4) the exogenous genes may be transferred into the nucleus without integration and may be expressed transiently [15].

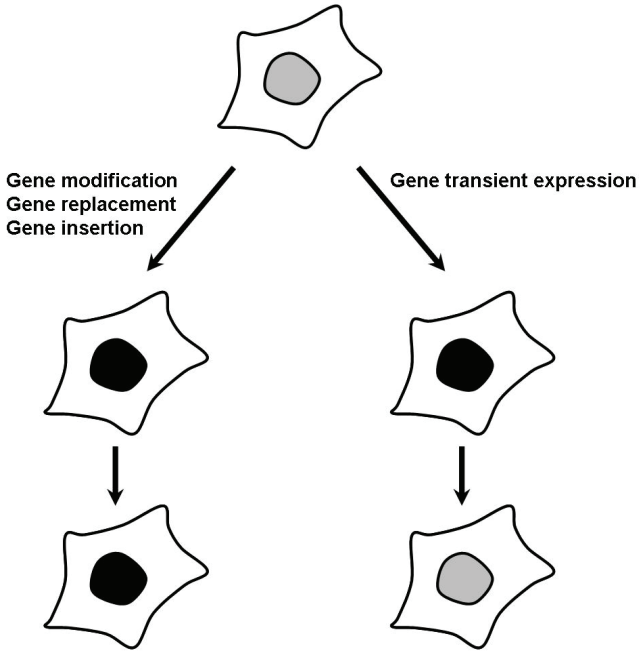

Figure 2. Functions of transferred genes. Gene modification, gene replacement and gene insertion lead to long-term gene expression, while the delivery of exogenous genes which temporarily stay in the nucleus induces transient expression.

Although large progresses have been made in the study of gene therapy, some hurdles are still standing on the way to achieve completely successful gene therapy. Several issues should be inevitably considered when gene therapy is applied, including safety, gene transfer efficiency, site-specificity and cost etc. Among these, the efficient transfer of therapeutic genes into the target cells is the first challenge for the researchers working in the field of gene therapy.

Today, numerous gene delivery systems and gene vectors/carriers have been invented and developed. They can enhance the gene transfer efficiency by different mechanisms, such as speeding up intracellular uptake, enhancing cells targeting, protecting DNA from enzymes, assisting DNA escape from lysosome and facilitating

DNA's nuclear entry etc. It has become increasingly clear that the success or failure of gene therapy critically depends on the development of gene carriers and gene transfer systems or techniques [18-21].

In general, currently used gene transfer methods can be divided into two categories: viral method and non-viral method, depending on whether virus vectors are involved. Numerous studies about these two gene transfer methods have been done, and each of them presents distinct advantages and weaknesses. Viral method offers high transduction efficiency and long-term gene expression, but it may be associated with toxicity, immunogenicity, carcinogenicity, poor target cell specificity, inability to transfer large size genes and high costs [15, 22-24]. In contrary, non-viral method offers the advantages of relative safety, ability to transfer large size gene, less toxicity, site-specificity and easiness for preparation, but it has the limitations of low transfection efficiency and poor transgene expression [25-28]. In short, neither of these two gene transfer methods is ideal, and their merits and/or shortcomings complement each other.

1.2 Viral gene delivery

Viral gene delivery is performed by using viruses that can bind to the host and introduce their genetic material into the cells (Figure 3). The viruses must be modified by deleting one or several viral structural genes and introducing the therapeutic genes before they can be used for gene therapy. The principle of this modification is to remove the genetic sequences that mediate viral replication and pathogenicity, to retain those required for viral binding, entry and gene delivery and to construct new therapeutic genes [15, 19, 29]. After this modification, the viruses used as viral vectors can not replicate thus can not cause diseases, while they still remain the capability to deliver exogenous DNA into cells. Normally, viral vectors contain strong promoters to allow high yield of transgene expression. Viral vectors with tissue-specific promoters can have transduction specificity which excludes the transgene expression in other cell types than the target cells. Some other strategies for virus mediated targeting transduction include the modification of the viruses' surface structures by specific recognition sequences which can allow the infection of specific cells or tissues. Nowadays, viral vectors falling into several categories have been studied. Each of them shows specific benefits and limitations, as reviewed by Boulaiz H. et al. (Table 1) [15].

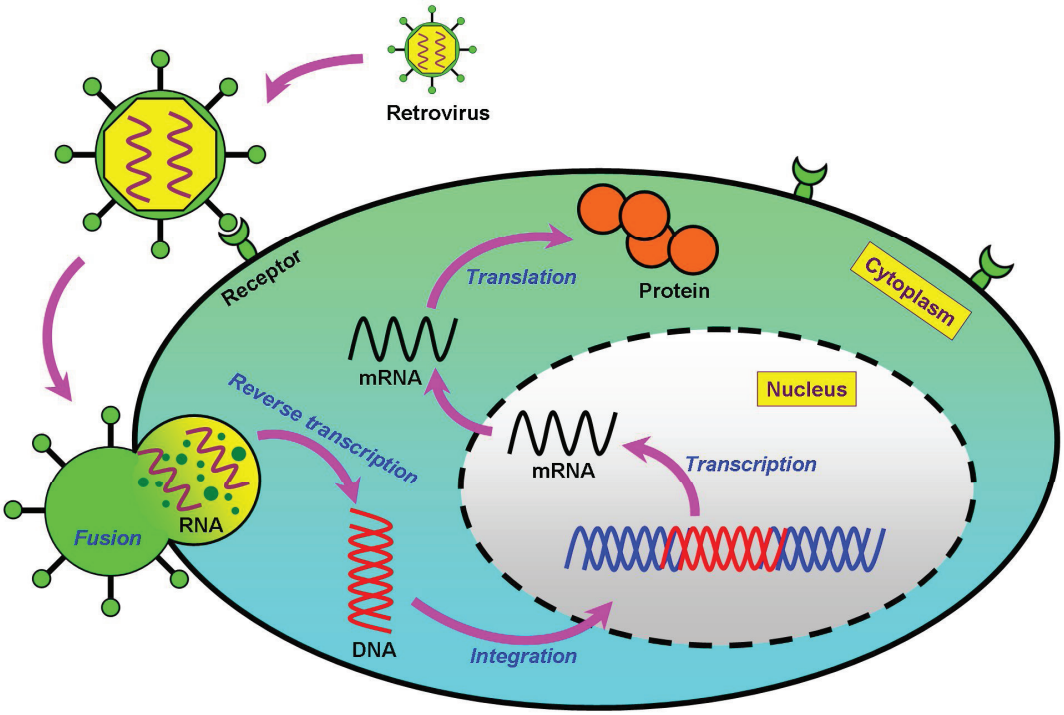

Figure 3. Retrovirus mediated gene delivery. The genetic material in retrovirus exists in the form of RNA molecules, while the genetic material of the host cell exists in the form of DNA. After infection of the host cell, retrovirus introduces its RNA together with some enzymes (reverse transcriptase and integrase) into the cell. During the process termed reverse transcription, the DNA copy from the RNA molecules of the retrovirus is produced by reverse transcriptase. After that, this DNA copy could be incorporated into the genome of the host cell, which is carried out by integrase. Finally, the successfully transduced gene will be stably expressed.

	Retrovirus	Adenovirus	Adeno-associated virus	Herpesvirus	Vaccinia virus
Nucleic acid	**RNA**	**DNA**	**DNA**	**DNA**	**DNA**
Particle size	**100nm**	**80-120nm**	**20-30nm**	**120-300nm**	**186nm**
Packaging capacity	**Up to 4-8 kb**	**4-8 kb**	**Low < 4 kb**	**High > 30 kb**	**25-75 kb**
Host range	**Dividing cell only**	**Dividing and non-dividing cells**	**Dividing and non-dividing cells**	**Dividing and non-dividing cells**	**Dividing and non-dividing cells**
Transgene expression level	**Moderate**	**High**	**Moderate**	**Moderate**	**Moderate**
Genome integration	**Yes**	**No**	**Yes**	**No**	**No**
Transgene expression stability	**Stable**	**Transient**	**Transient/Stable**	**Transient**	**Transient**

Table 1. Viral vectors used for gene therapy

1.3 Non-viral gene delivery

Just as its name implies, non-viral gene delivery refers to any virus-free methods for gene transfer. DNA used in non-viral gene delivery can be delivered into cells by physical forces (physical methods) or by synthetic or nature compounds (chemical methods). Compared with viral counterparts, non-viral methods generate less toxicity and immunogenicity. Moreover, cell-specific gene transfer could be realized easier via non-viral methods, since physical delivery allows precision of spatial control and chemical carriers provide the opportunities to be modified by some site-specific ligands. Other merits of non-viral methods include low cost for production, potential for repeat administration and the ability to transfer DNA with large size.

Generally, non-viral methods show less transfection efficiency than viral methods, and in most cases, the transgene expression is insufficient in terms of time duration. However, some recent studies have indicated that gene transfer by some non-viral methods could achieve the transfection efficiency and expression duration on a clinically meaningful level. Indeed, non-viral gene delivery has already been applied in many clinical trails such as cancer gene therapy [30, 31] and the treatment of cardiovascular diseases [32].

1.3.1 Barriers for non-viral gene delivery

The ideal process of non-viral gene delivery is thought to be that nucleic acid is rapidly delivered to the cell population of interest, is quickly uptaken by the cells, is subsequently transported into the appropriate cellular compartment in which the functionalization of the nucleic acid takes place. For this reason, several extracellular and intracellular barriers must be conquered to ensure the effective gene delivery. Epithelial, endothelial cell linings and the extracellular matrix (ECM) surrounding the cells compose the anatomical barriers to prevent nucleic acid macromolecules from accessing to the target cells directly. DNA-loaded particles administrated via blood circulation can be cleared by some phagocytes, such as Kupffer cells in the liver and residential macrophages in the spleen, before they reach the target cells. After systemic administration, free nucleic acid molecules without protection can be fast degraded by various nucleases existing in blood or ECM [33].

Crossing cell membrane is regarded as the most restricting step for non-viral gene delivery. Normally, nucleic acids aren't able to pass through the plasma membrane, because of the nature repulsion between the nucleic acids and the cell surface since both of them are negatively-charged. Nevertheless, some methods have been proved to facilitate the cellular entry of nucleic acids. Physical methods such as gene gun, electroporation and sonoporation can produce transient holes on cell membrane that allow the free entry of nucleic acids [34]. Chemical methods condense and pack nucleic acid molecules via chemical compounds such as cationic lipids or cationic polymers, thereby form complexes which present positive surface charge and could be easily uptaken by cells via endocytosis, pinocytosis or phagocytosis [35].

After cellular uptake, endosomes containing DNA will transform into digestive lysosomes. This transforming process consists of two steps: first is the maturation of endosomes from "early" to "late" stage, and second is the fusion of mature endosomes with lysosomes [36]. DNA in endosomes will eventually be degraded by lysosomal hydrolytic enzymes unless it can escape from the endosomes before the endosomes become mature. Currently, two endosomal escape mechanisms have been explored. First, pH-responsive amphipathic peptides or lipid components with acid sensitive bond are involved. They can disrupt the endosome membrane, and thus facilitate the endosomal escape [37, 38]. Second, cationic polymers such as polyethylenimine (PEI) were used to condense DNA and help DNA to escape from endosomes through the process named "proton sponge effect" [39]. PEI is only partially protonated at neutral pH, which allows remaining nitrogens to be further protonated at lower pH inside the endosomes. This will induce the influx of chloride counter ions, thereby cause osmotic pressure within the endosomes, and finally trigger the swelling and rupture of endosomes [40]. The "proton sponge effect" seems critically dependent on the nitrogens that are protonatable at lower pH. One evidence is poly-L-lysine (PLL). PLL has only primary amine groups that can not be further protonated at lower pH value, and thus shows less transfection efficiency than PEI.

After being released from endosomes into cytoplasm, DNA in free form or as complex has to be transported into nucleus where transcription happens. Although the mechanism of such transport process is still poorly understood at present, some studies have revealed that the complexasion by cationic lipids or cationic polymers could protect DNA from degradation by cytoplasmic nucleases, and thus improve the opportunity to enter nucleus [41]. Recently, a novel concept has been proposed, in which

a microtubule-directed transport mechanism was involved for intracellular transport of DNA-loaded nanoparticles [42].

Being similar to cytoplasm membrane, the nuclear envelope is another crucial barrier for non-viral gene delivery which prevents DNA from entering the nucleus. The nuclear envelope is a double-layers membrane and is interrupted by nuclear pore complexes (NPC) which control the transport through the nuclear envelope. NPC has very small diameter (~9nm) allowing the free diffusion of molecules with small or medium size, but restricting the free entry of large macromolecules into the nucleus [43]. In nuclear transport, the uptake of large molecular proteins is an active process, which is mediated by nuclear localization signal (NLS) peptide through sequence-specific recognition [44]. The modification of gene carriers with NLS showed enhanced gene delivery, which is presumably due to the improved nuclear entry [45, 46]. Another possibility to increase the nuclear entry is to modify plasmid DNA. In one successful example, SV40 sequence was included into plasmid DNA and such modification led to increased transgene expression, especially in non-dividing cells [47]. The SV40 enhancer is a region known to bind to a number of general transcription factors. In cytoplasm, protein/DNA complexes can be formed through the binding of SV40 onto transcription factors; and subsequently, these complexes will enter the nucleus through the protein import machinery [48, 49]. Nuclear entry of DNA is largely dependent on cell cycle. The dissolution and reorganization of nuclear envelope during or close to mitosis can largely facilitate the nuclear entry of DNA molecules [50]. This has been confirmed by several studies, in which cells in S-phase and G2/M-phase showed significantly higher transfection efficiency than cells in G1-phase [51-54]. This cell cycle dependent property holds high potential for cancer treatment, because of the high proliferation rate of tumor cells.

In non-viral gene delivery, only very small fraction of DNA is finally delivered into nucleus, while most part is gathered in the perinuclear granular region [55]. The observation of intact polymer (e.g. PEI)/DNA complexes in the nucleus has been reported, indicating that the separation of DNA molecules from the polycations is not an indispensable procedure before nuclear entry [56, 57].

1.3.2 Physical methods

Microinjection

Microinjection refers to the process of using a micropipette to inject solutions directly into a single living cell at a microscopic or borderline macroscopic level [58, 59]. Barber M.A. first described this technique which forms the basis for today's microinjection applications [60]. Microinjection is a relative simple, economic, effective, reproducible and non-toxic method. Normally, a needle with the diameter around 0.5-5μm was used to penetrate the cell membrane and/or the nuclear envelope and inject the genetic materials. It can be used to transfer large size DNA. However, microinjection requires the individual manipulation of each cell, which largely restricts the efficiency of performance. Other drawbacks include the low level and short duration of transgene expression. Microinjection can be used in vaccination procedures, in which the transgene expression is only required at a low level to induce an immunological response.

Needle injection

The localized needle injection of naked DNA into mouse muscle was first demonstrated by Wolff J.A. et al. in 1990 [61]. After that, it has been applied onto various tissues including liver, skin, brain and tumors etc. Needle injection is thought to be the simplest and safest gene transfer approach by which the therapeutic genes can be directly injected into the tissues, organs or blood streams in a simple manner [62, 63]. Furthermore, not being limited by naked DNA, needle injection can be used to transfer RNA, DNA/cationic polymer (or cationic lipid) complex and oligonucleotide. Some other injectable agents can been involved into this procedure to enhance the gene expression, such as transferrin, water-immiscible, solvents, nonionic polymers, surfactants or nuclease inhibitors [64-67]. Owning to these merits, this gene transfer procedure is particularly attractive for the clinical applications. A lot of efforts have been made in cancer gene therapy using this approach [68-70]. Injection of vascular endothelial growth factor-2 (VEGF-2) gene into patients suffering chronic myocardial ischemia has shown some positive therapeutic effects including the improvement of heart function. [71]. Today, direct injection of genes into muscle or skin has become a very convenient and useful tool to evaluate DNA-based vaccination [33]. The disadvantages of needle injection include the poor level of transgene expression, especially when naked plasmid DNA was injected since unprotected DNA will be rapidly degraded by the nucleases.

Jet injection

Compared with conventional needle injection, jet injection is a needle-free gene delivery method that was first described in 1947 [72]. In jet injection, DNA solution is driven by pressurized gas, usually CO_2, to form high-speed and ultrafine stream. Generally, the procedure of jet injection consists of several steps: DNA loading, gas pressure adjustment and injection. The high-speed DNA stream hitting the target cells generats pores on cell membrane. The intracellular entry of DNA can be largely facilitated by these pores. In jet injection, the mechanical properties of the target cells should always be considered, and the gas pressure should be adjusted to fit the cells. Ren S. et al. reported that transgene expression by jet injection could be improved around 50-folds compared with needle injection [73]. Jet injection can be used to transfer genes into various types of cells or tissues, such as muscle, skin and fat. It has shown high potential for cancer inhibition [74, 75]. Most importantly, no serious side effects were reported until now except for local pain, edema and site-bleeding [76].

Gene gun

Gene gun, also known as Ballistic DNA injection or DNA-coated particle bombardment, was originally designed for plant transformation. Today, this technique has been widely used as a gene delivery approach owning to its numerous merits. It has been applied on various tissues or cells including skin, mucosa, muscle, tumors and some surgically exposed tissues [77, 78]. The particles used as payload are heavy metals, usually gold, tungsten or silver. These particles are first coated with plasmid DNA, then are accelerated by electric discharge or gas jet to a certain speed, and finally fired at the target cells or tissues. The momentum of the particles can lead to the penetration into the tissues around a few millimeters deep, and thereby the loaded DNA can be released into cells on the path of the particles [79]. Particle speed, particle size and dosing frequency are crucial parameters that influence the particle penetration, the tissue injury degree and the gene transfer efficiency [80]. As a simple and effective approach for gene delivery, gene gun has extensively been tested for intramuscular, intradermal, and intratumor genetic immunization. It was reported that gene gun can induce more immune response with lower doses than needle injection in large animal models and clinical human trials [33]. Further improvement of this approach could be made from the following aspects: 1) modification of the particles' surface to allow higher DNA loading capacity, 2) precise control of particles flying and DNA release, 3) the shortening of

operation time duration, and 4) reduced tissue damage without decreasing gene transfer efficiency.

Electroporation

Gene delivery by electroporation employs high-voltage electrical currents to create transient nanometric pores on cell membrane, thus allowing negatively-charged DNA to move intracellularly and to remain trapped within the cells. The first utilization of electroporation for *in vitro* and *in vivo* gene transfer was reported in 1982 [81] and 1991 [82], respectively. Until now, numerous works have been done for deeper study on electroporation including the *in vitro* optimization [83] and *in vivo* test in different types of tissues [81, 84]. Hasson E. et al. found that electroporation could significantly enhance the transgene expression in lung cells which were cultured *ex vivo* [85]; Dean D.A. et al. reported the application of this technique in living animals by placing electrodes into the chest [86]; Magin-Lachmann C. et al. successfully transfered large size DNA (100kb) into muscle cells [87]; Molnar M.J. et al. observed that gene expression lasts over 1 year in mouse muscle after gene transfer via electroporation [88]; and Marti G. et al. studied *in vivo* electroporation to improve wound healing in a diabetic mouse model by transferring keratinocyte growth factor-1 (KGF-1) [89]. Excitingly, localized gene transfer by electroporation was reported by Sakai M. et al. [90]. In their study, systemic plasmid DNA injection through the portal vein followed by a localized electroporation on rat liver resulted in widespread gene expression in hepatocytes in the treated lobe but not in the surrounding lobes. This indicates the possibility that DNA can be administrated via blood circulation and then be locally delivered in defined tissue via electroporation. The gene transfer efficiency of electroporation is influenced by several factors including the electrical current intensity, time interval between discharges, concentration and type of DNA. It was also reported that the age of the recipient animals [91] and the distribution of plasmid DNA in the tissue [92] could influence transfection efficiency. Electroporation is relatively safe, efficient and reproduceable. Generally, it can be applied to all cell types. With optimized parameters, electroporation could achieve high transfection efficiency being similar to viral method [93]. Nevertheless, some drawbacks of this approach still exist, especially for *in vivo* application. The limited range between the electrodes (~1cm) restricts the gene transfer to large area of tissues. Furthermore, a surgical procedure is required for *in vivo* electroporation to put the electrodes into the organs. Moreover, high voltage applied to

cells might induce tissue damage and probably influence the stability of genomic DNA [94, 95]. However, some of the concerns may be resolved by further technical development including new design of the electrodes and optimization of the operating parameters (e.g. the frequency and duration of electric pulses).

Sonoporation

Sonoporation, also called ultrasound-facilitated gene transfer, as the term indicated, is a technique that uses ultrasound waves to induce cell membrane permeabilisation and thereby realize gene transfer. It was first described in 1954 by which the transdermal penetration of drugs was enhanced by ultrasound [96]. Currently, ultrasound has been used for gene transfer in cellular [97] and tissue levels [98], expanding the methodology of physical gene transfer methods. Several critical parameters determine the transfection efficiency of sonoporation, including the ultrasound frequency, the ultrasound intensity, the duration of the ultrasound applied, the amount of plasmid DNA used and the tissue type. Normally, ultrasound with frequency 1-3MHz and intensity 0.5-2.5W/cm^2 was selected for gene delivery studies [97]. With the facilitation of ultrasound, a significant enhancement (10-20 folds) of reporter gene expression could be achieved over that of naked DNA [79]. The use of contrast agents or some conditions that make cell membrane more fluidic can largely enhance the gene transfer efficiency [99-101]. The contrast agents are normally air-filled microbubbles stabilized by surface active molecules such as albumin, polymers or phospholipids. These microbubbles expand and shrink rapidly under ultrasound irritation, releasing local shock waves that transiently disrupt the membrane of nearby cells and consequently facilitate local gene transfer. The utilization of complexes composed of DNA/cationic lipids or polymers could further improve the gene transfer efficiency, which has been studied both *in vitro* and *in vivo* [102, 103]. Sonoporation shows the advantages of safety, noninvasiveness and the ability to transfer genes into internal organs without surgical operations [104-107]. Interestingly, recent study demonstrated that ultrasound could enhance the permeability of blood-brain barrier [108]. However, like other non-viral methods, the gene transfer efficiency of sonoporation needs to be further improved.

Hydrodynamic gene transfer

Hydrodynamic gene transfer can deliver genes into highly perfused internal organs. When a large volume of DNA solution is rapidly injected into mouse tail vein,

transgene expression can be observed in liver, lung, kidney, spleen and heart. It was reported that an injection of 5μg of plasmid DNA could finally generate around 45μg luciferase protein per gram liver tissue, and approximately 30-40% of hepatocytes could be transfected [109, 110]. The high pressure is the DNA driving force. When the large volume of DNA solution (more than 8% of body weight) is injected quickly (around 5 seconds or less) into the tail vein, a transient overflow of injected solution will happen at the inferior vena cava exceeding the cardiac output. As a result, a reversible permeability change in the endothelial lining will be induced and some transient pores in hepatocyte membrane will be generated, which facilitates the entry and expression of DNA [111].

Hydrodynamic gene transfer has been used in many rodent models to transfer therapeutic genes including hemophilia factors [112, 113], alpha-1 antitrypsin [109, 114, 115], cytokines [116], hepatic growth factors [117] and erythropoietin [118] etc. Importantly, this approach can deliver not only genes but also other water soluble compounds like small dye molecules, proteins, oligonucleotides and siRNAs etc [119]. The delivery efficiency is highly dependent on organ type, injection volume, injection speed and the total amount (or concentration) of the functional substance. At present, hydrodynamic gene transfer can not be applied in human clinical trials because of the injection volume. Mouse or rat can be treated with an injection volume equivalent to 8% of its body weight, which would be far beyond the acceptable level if the same ratio of injection volume was applied to human. However, by using a catheter-based technique, hydrodynamic gene transfer into the liver of pigs has been carried out with reduced liver damage [120-122]. The development of new technologies, such as computer-controlled delivery systems, could evolves this method for further utilization in clinical applications [123].

Mechanical massage

This method was reported by Liu F. et al. [124]. Their result showed that significant gene expression in the liver of mice could be achieved via simple mechanical massage after intravenous injection of naked plasmid DNA. It is believed that mechanical massage can generate transient disruption on the membrane of liver cells, which allows the entry of plasmid DNA by diffusion. They also found that the level of liver gene expression is significantly related with the venous blood pressure, suggesting that liver

gene transfer by mechanical massage is, at least in part, due to pressure-mediated effect [125].

1.3.3 Chemical methods

In recent years, chemical non-viral vectors, such as calcium phosphate, diethylaminoethyl-dextran, cationic lipids and cationic polymers, have been widely studied due to their advantages including safety, large size gene transfer capability, less toxicity and easiness for preparation etc [15]. Among these, cationic lipids and cationic polymers show prospects to be promising gene carriers by forming condensed complexes with negatively-charged DNA through electrostatic interactions. The condensed complexes with positive net charge can be taken up by cells via endocytosis. Polymers or lipids can facilitate endosomal gene escape and protect DNA from degradation by nuclease. Finally, a small fraction of DNA can be released into cytoplasm and migrate into the nucleus where transgene expression takes place [79, 126].

Cationic lipids (liposomes)

The first utilization of cationic lipid was reported in 1987 by Felgner P.L. et al. who used a double chain monovalent quaternary ammonium lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), to condense and transfer DNA into cultured cells [127]. After that, numerous cationic lipids have been developed and studied [128-132].

All cationic lipids are composed of three parts: hydrophilic head group, linker and hydrophobic anchor (Figure 4) [133]. The hydrophilic heads normally employ one or more positively-charged amine groups as the cationic moiety. According to the charge number on the hydrophilic head, cationic lipid can be classified as monovalent and multivalent. The hydrophobic anchors are nonpolar hydrocarbon moieties of the cationic lipids. They can be grouped into several categories according to the chemical structure: single chain hydrocarbons [134], double-chain hydrocarbons [135], cholesterol [136] and vitamin D-based [137]. The linker is a chemical part connecting the hydrophilic head group and the hydrophobic anchor. The linkers also play very important role in cationic lipid mediated gene delivery because their properties determine the biodegradability of cationic lipids and influence the toxicity and gene transfer efficiency [138, 139].

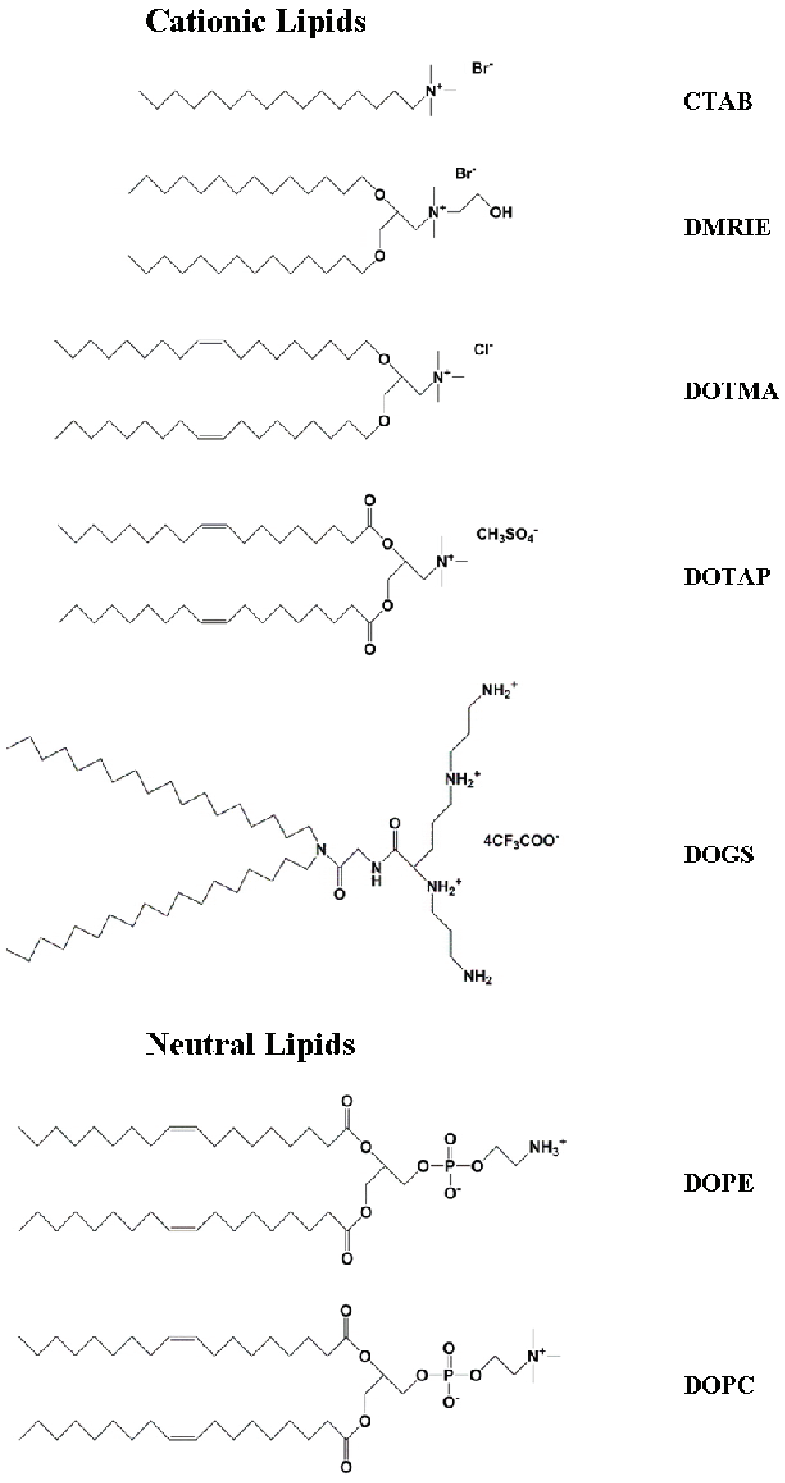

Figure 4. Structure of some cationic lipids and neutral lipids (co-lipids) commonly used in gene therapy.

Cationic lipids can be used alone or together with co-lipids. 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE), one of the most commonly used co-lipids, led to the improvement of gene transfer efficiency when mixed with some cationic lipids, such as DOTMA and DOTAP (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-

trimethylammonium methylsulfate) [140-142]. This effect of DOPE was due to its capability to facilitate the lipoplex forming and its tendency to transit lipoplex from a bilayer to a hexagonal structure under acidic pH at endosomal level, which may facilitate the fusion or destabilization of endosomal membranes [143-146]. Further studies also found that DOPE could facilitate the DNA release from lipoplex and the DNA escape from endocytotic vesicles [147, 148]. Cholesterol is another commonly used co-lipid. Compared with DOPE, cholesterol could form more stable but less efficient lipoplex. This is meaningful for *in vivo* gene delivery since cholesterol could stabilize the lipoplex against the destructive effects of serum, and thus provide better biological activity than DOPE [149-153].

The lipoplexes can be prepared by mixing diluted plasmid DNA solution and cationic liposomes. Several lipoplex structures have been reported including the "spaghetti-meatball", "sandwich", "honeycomb" and "invaginated bilayer" etc [144, 154-156]. The transfection efficiency of lipoplexes was influenced by several factors, including the structure and property of cationic lipid, the lipoplex size [157-159], the charge ratio between the cationic lipid and DNA [160, 161], the applied lipoplex amount, the structure and proportion of co-lipid [162-164], the cell type [165] and the cell cycle [166].

In summary, as non-viral gene delivery vectors, cationic lipids show advantages of being inexpensive and easy to prepare. They can also be modified for targeted gene delivery. However, two main shortcomings of cationic lipids still need to be solved, i.e. the toxicity and relative low transfection efficiency, to extend their applications especially for *in vivo* treatment.

Cationic polymers

Cationic polymers are used as gene carriers since they can largely improve gene transfer efficiency. Generally, cationic polymers possess amine groups at a high density. These amine groups are protonatable at neutral pH and form positively-charged polymer molecules. When cationic polymers are mixed with negatively-charged DNA, polymer/DNA complexes (polyplexes) are generated through the electrostatic interaction. Polyplexes are the transfection units having the nanoscale particle size (normally form dozens to hundreds of nanometers). It's believed that two mechanisms contribute to the improvement of gene transfer efficiency in cationic polymer mediated gene delivery. First, polymer can enhance the polyplex uptake via endocytosis because there are charge-charge interactions between polyplexes and the anionic sites on cell

surface. Second, polymer can protect DNA from nuclease degradation and facilitate DNA's endosomal escape (Figure 5).

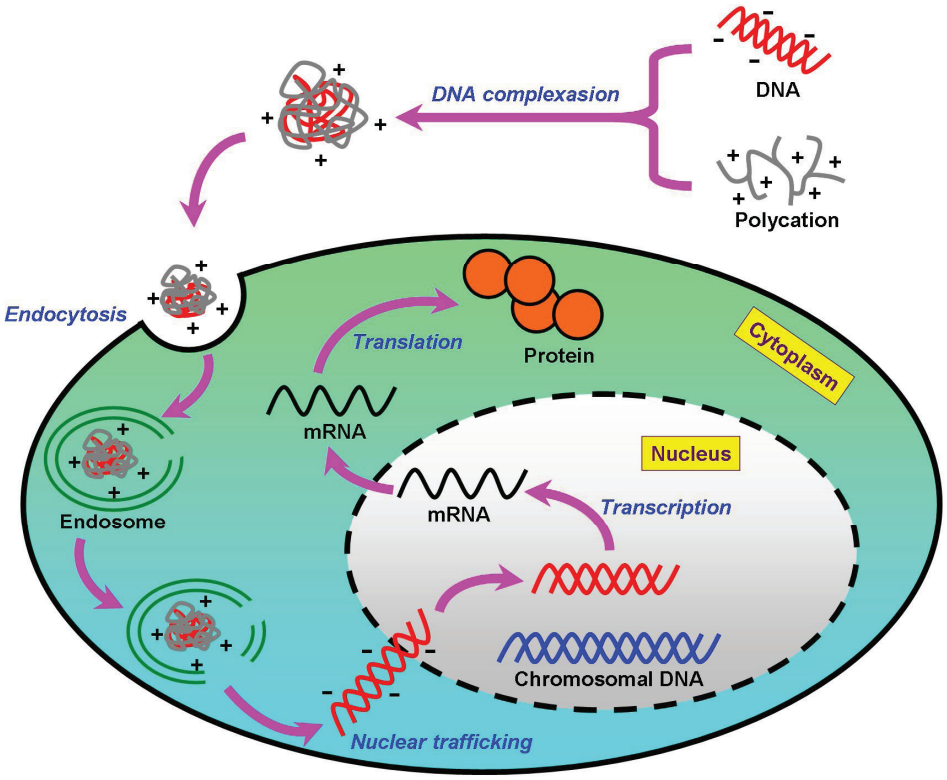

Figure 5. Cationic polymer mediated gene delivery.

In the past years, a large number of cationic polymers have been developed and studied (Figure 6). These include: 1) natural polymers such as chitosan [167, 168], 2) dendrimers such as polyamidoamine (PAMAM) [169], 3) polypeptide such as PLL [170], polyarginine [171], polyornithine [172, 173], histones [174] and protamines [175] and 4) other polymers such as PEI [176, 177] and polyphosphoester (PPE) [178], etc. Additionally, some of them have been modified to improve the functions, such as increasing transfection activity or reducing toxicity. As a result, some polymers have a large number of derivatives. However, with the usage of different polymers, the transfection activity and toxicity might vary dramatically.

Among numerous cationic polymers, PEI has been considered as the most effective one. PEI can be synthesized via acid-catalyzed ring opening polymerization of aziridine as either branched or linear structure, or via hydrolysis of poly(2-ethyl-2-oxazolium) as linear structure. Branched-PEI (B-PEI) contains primary, secondary and tertiary amine groups, while linear-PEI (L-PEI) mostly has secondary amines except the primary amines of the terminal ends. PEI is capable to condense DNA molecules to form

PEI/DNA complexes. These complexes are homogeneous spherical particles that can be uptaken by cells via endocytosis [179, 180]. Intracellularly, PEI's higher charge density can provide protection for DNA against nuclease degradation, and can facilitate DNA's endosomal release through "proton sponge effect" [39].

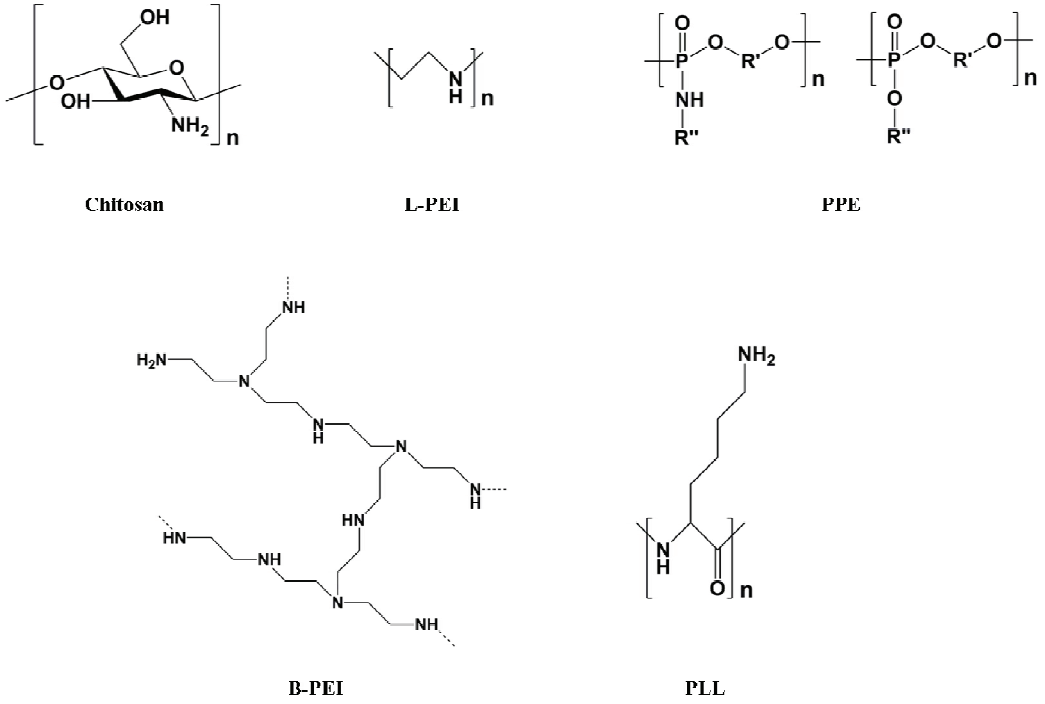

Figure 6. Structure of some cationic polymers commonly used in gene therapy.

The performance of PEI in gene delivery is critically determined by its molecular weight. With the increase of PEI's molecular weight, the gene transfer efficiency is increased, whereas the cytotoxicity is also improved [181]. Hence, the balance between the efficiency and toxicity is the most important point for PEI mediated gene delivery. Currently, 25kDa (molecular weight 25,000 Dalton) B-PEI and 22kDa L-PEI are the most commonly used PEI polymers. Both of them lead to efficient gene transfer efficiency and induce moderate cytotoxicity [182, 183]. L-PEI leads to faster gene expression than B-PEI, perhaps due to the weaker DNA condensing capability of L-PEI that allows faster polymer/DNA dissociation in the cells. *In vivo* study has shown promising result, in which L-PEI was used for intravenous gene delivery into mouse lung [184]. Furthermore, L-PEI has been shown to mediate a cell cycle independent gene delivery [52], providing an option to deliver genes into slow-dividing cells.

In our work, 25kDa B-PEI mediated gene delivery into bone marrow derived human mesenchymal stem cells (MSCs) was studied [Attached article 1]. Our results indicated

that PEI has the potential to become a clinical meaningful non-viral gene vector, though further improvement is still necessary to enhance its gene delivery performance. In another study, we investigated a gene activated substrate (GAS) mediated non-viral gene delivery [Attached article 4&5]. GAS solution was prepared by mixing substrate materials (rat tail collagen or human fibronectin) with 25kDa B-PEI/DNA complexes. The GAS solution could be easily coated onto cell culture dish or the surface of scaffold materials. After the drying of GAS, the cells could be cultured on it and be transfected subsequently (Figure 7). Compared with normal transfection, in which polyplexes were added directly into cell culture medium, GAS mediated gene delivery could lead to lower cytotoxicity, sustained gene release, localized gene delivery and relative high transfection efficiency. In addition, such GAS could be easily coated onto scaffold materials for implantation, and *in vivo* transgene expression has been observed.

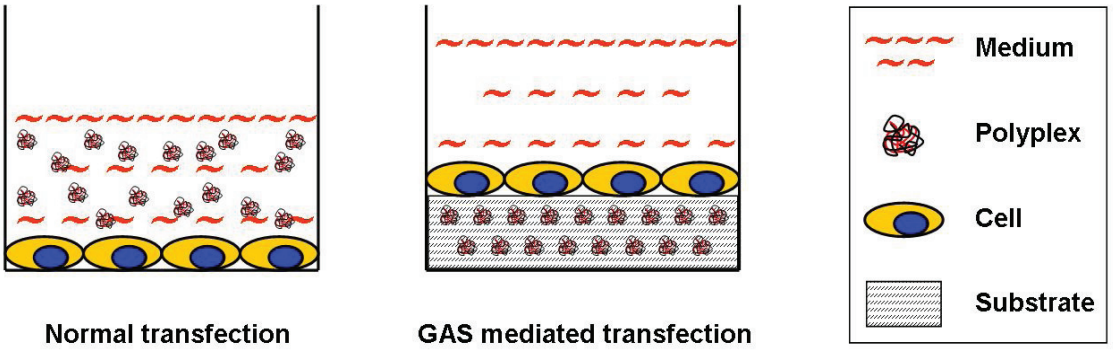

Figure 7. Principle of *in vitro* transfection via normal method and GAS mediated method. In normal transfection, polyplexes were added directly into cell culture medium. In GAS mediated transfection, the procedure consists of several steps: 1) the polyplexes were mixed together with substrate materials; 2) the GAS mixture was coated onto cell culture dish; 3) after drying of GAS, cells were cultured onto it; 4) sustained gene release and transfection were achieved in the following days.

Recently, some strategies to prepare novel polymers based on low molecular weight-PEI (LMW-PEI), such as PEI800 (molecular weight 800Da) and PEI1800 (molecular weight 1800Da), have been developed. LMW-PEI could be crosslinked via some crosslinking reagents or inert polymers. After such crosslinking, the new polymers present the advantages of both high and low molecular weight-PEIs, i.e. high transfection efficiency and low cytotoxicity. Meaningfully, biodegradable polymers are available if biodegradable crosslinking bonds or biodegradable polymers are involved [185-188]. Since the main drawback of high molecular weight PEI is the non-

biodegradability, biodegradable crosslinking will largely extend the application of PEI-based gene delivery especially for *in vivo* treatment.

Despite the achieved progress, cationic polymers need to be further studied to improve their performance. Potential strategies include: 1) modification or conjugation by other polymers, targeting ligands or nuclear localization signals, 2) combination with other gene carriers such as liposomes and inorganic materials, and 3) synthesis of novel polymers.

Inorganic nanoparticles

Inorganic nanoparticles show potential to become gene carriers since they can be loaded with nucleic acids via absorption or conjugation, and the loaded nucleic acids can be transfered into living cells when these nanoparticles are uptaken by cells. Compared with organic nanoparticles, inorganic nanoparticles hold some advantages, such as high stability, low cytotoxicity and easiness for preparation. Numerous inorganic nanoparticles have been studied for gene delivery, including calcium phosphate [189], carbon nanotubes [190], magnetic nanobeads [191], silica [192], gold [193], quantum dots [194], and double hydroxide [195] etc. Some of them showed high promising performance. For example, by conjugating DNA onto supraparamagnetic nanoparticles, "magnetic force guided" gene delivery was realized both *in vitro* and *in vivo* [196, 197]. By immobilizing plasmid DNA onto the nickel-embedded carbon nanotubes and applying a magnetic driving force, an unprecedented high transfection efficiency was achieved [198].

2. Stem cell therapy

Adult stem cells, also known as somatic stem cells, are undifferentiated cells holding the properties of self-renewal and multipotency. They are found throughout the body after embryonic development, that can replenish dying cells and regenerate damaged tissues through multiplication via cell division. The ability to generate the cells of the organ from which they originate makes adult stem cells attract scientific interest. More importantly, unlike embryonic stem cells, the use of adult stem cells in research and therapy does not induce ethical controversy since they are derived from adult tissues.

2.1 Mesenchymal stem cell

Mesenchymal stem cell (MSC) is an important cell type of adult stem cells. MSCs are multipotent and can differentiate into a variety of cell types, such as adipocytes, chondrocytes, muscles, osteocytes and stromal cells (Figure 8) [199, 200]. The first identification of MSCs was done about 30 years ago by Friedenstein A.J. et al. Since then MSCs have been isolated from bone marrow due to their ability to adhere to cell culture plastics [199]. Beside bone marrow, MSCs can be isolated from various other tissues including peripheral blood [201], periosteum [202, 203], umbilical cord blood [204], synovial membrane[205], pericytes [206], trabecular bone [207, 208], adipose tissue [209, 210], limbal stroma [211], amniotic fluid [212], lung [213], dermis [214] and muscle [215]. Currently, bone marrow aspiration is considered to be one of the most accessible and enriched sources of MSCs. Multipotent cells existing in bone marrow can gain access to various tissues via the circulation, subsequently start differentiation according to the requirements of maintenance and repair of a certain tissue type.

Due to the multipotency to differentiate into a various cell types, human MSCs have been a promising candidate for clinical use. The use of human MSCs in clinical applications requires the biological understandings of MSCs. Currently, the MSCs-based bench works focus on several aspects including the identification of MSCs, the *ex vivo* expansion, the senescence, the control of differentiation potential and the delivery method. The bedside application of MSCs in clinical therapy could be performed in several ways: local transplantation, systemic transplantation and combination with

tissue engineering. Some clinical case reports have demonstrated the use of MSCs in the treatment of bone defects [216], cartilage defects [217], myocardial infarction (MI) [218], chronic skin wounds [219], osteogenesis imperfecta in children [220], graft-versus-host disease [221], Hurler syndrome [222] and tissue reconstruction [223]. Recent study further indicated that MSCs can support unrelated donor hematopoietic stem cells and regulate immune response [224].

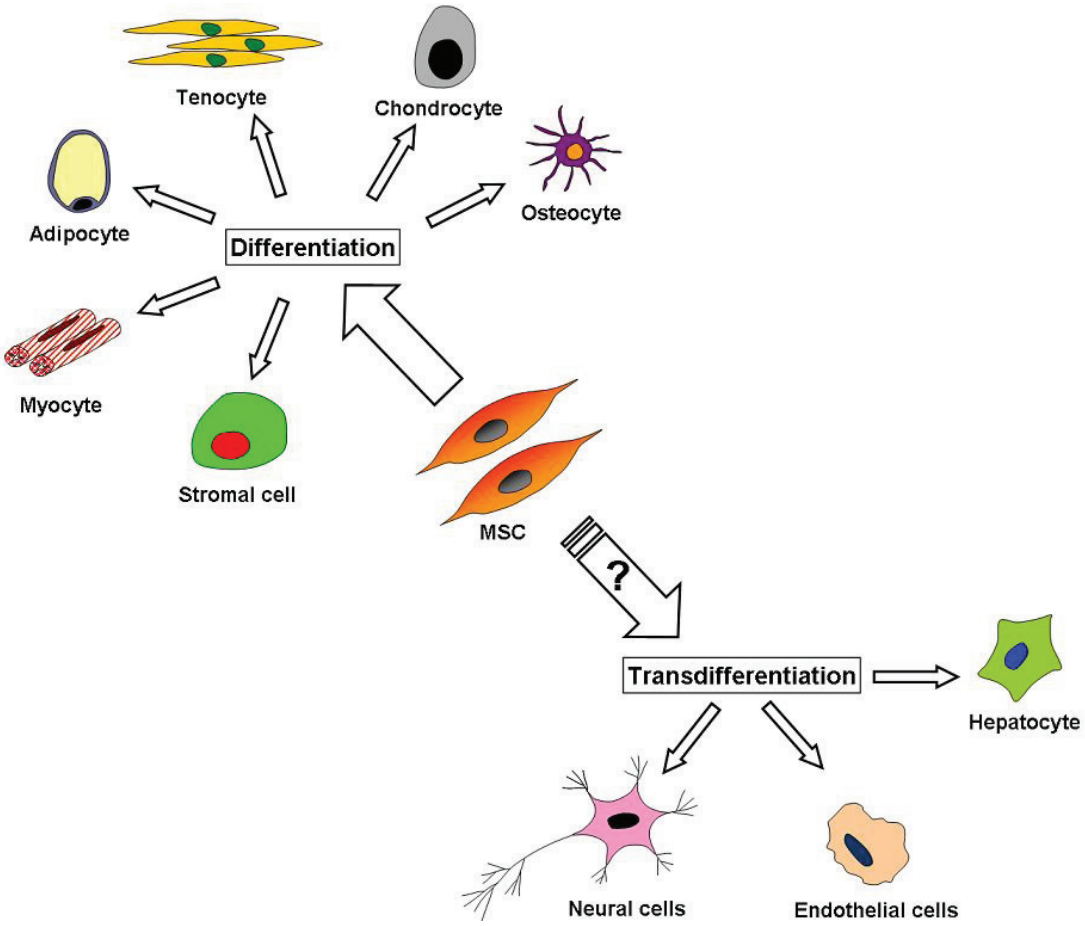

Figure 8. Models of MSC differentiation.

2.2 Hematopoietic stem cell

Hematopoietic stem cell (HSC) is a type of multipotent adult stem cells that are the source of all blood cell lineages, including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells) (Figure 9)[225, 226]. HSCs transplantations are most often performed for people with diseases of the blood, bone

marrow, or certain cancer. HSCs-based therapeutics have been applied for kidney repair [227], liver repair [228], multiple sclerosis treatment [229], beta-thalassemia treatment [230] and multiple myeloma remission [231] etc.

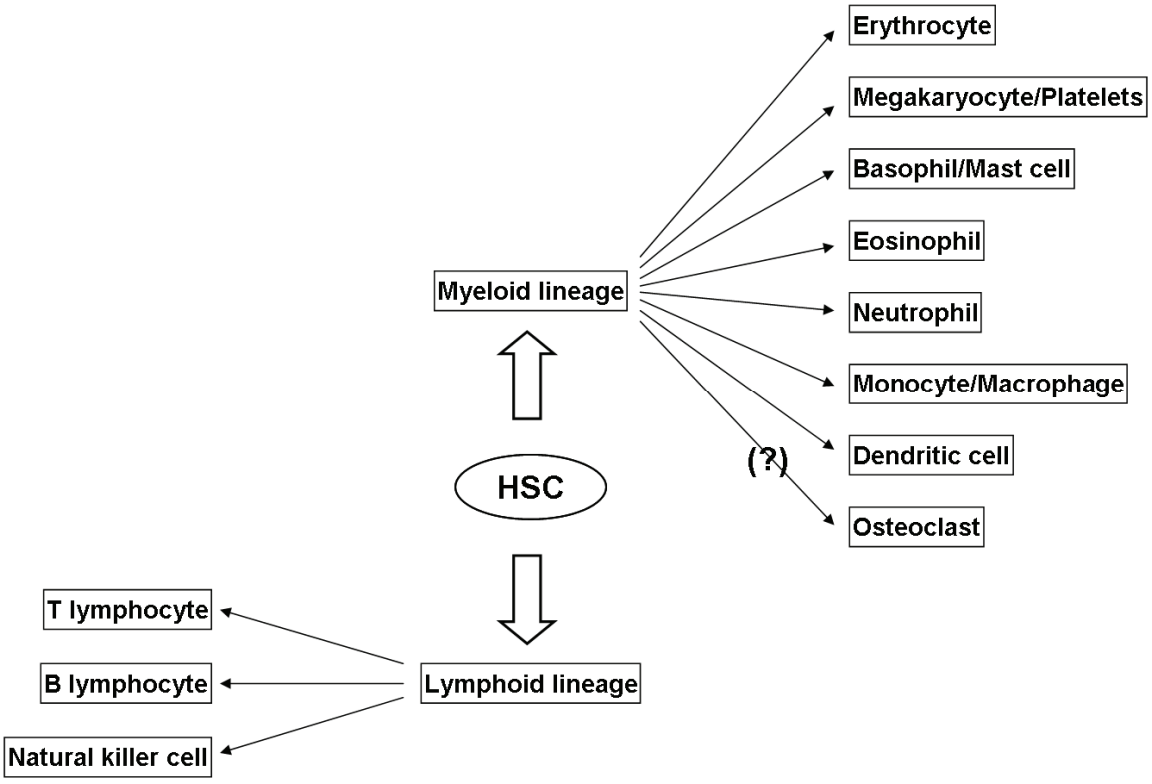

Figure 9. Models of HSC differentiation.

In our study, the therapeutic effect of stem cells on the restoration of heart functions after MI in a rat model was studied [Attached article 2]. Following acute MI, Matrigel was delivered into myocardium by intracardiac injection. We found that the left ventricular (LV) function, the infarct wall thickness of left ventricle and the capillary density of the Matrigel treated hearts were significantly improved, compared with the control group (PBS treated hearts). In addition, the number of $CD34^+$ and $CD117^+$ stem cells was found to be significantly more in the Matrigel treated hearts than in the PBS treated hearts. Thus, we assumed that the restoration of myocardial functions may partly attribute to the improved recruitment of $CD34^+$ and $CD117^+$ stem cells.

3. Application of gene delivery in stem cell therapy

Genetic modification of stem cells is an attractive approach for stem cell therapy because stem cells have higher proliferative capacity and long-term survival compared with other somatic cells. Genetically modified stem cells can deliver certain genes or proteins into organs or tissues according to specific requirements.

By genetic modification, stem cells could be guided to directed and complete differentiation towards the desired lineages. The fate of transplanted stem cells *in vivo* mainly depends on the microenvironment they home. However, not all transplanted cells differentiate into the desired lineages to help the repair of the damaged tissue. Recent study has indicated the potential risk of transplanted MSCs that differentiated into osteoblastes in the heart [232]. Thus, to guide the differentiation of stem cells by genetic modification with key differentiation factors seems crucial for stem cell therapy. Some studies based on animal models have shown that MSCs transduced with BMP2 and BMP4 could repair articular cartilage and bone defects since BMPs have the ability to induce chondrogenic and osteogenic differentiation [233-236]. More importantly, the genetically modified stem cells not only themselves undergo differentiation but also stimulate the neighbouring cells to participate in the repair process [200]. Furthermore, the therapeutic efficacy of stem cells could be improved via genetic modification. As we know, the clinical benefits of adult stem cells after transplantation are normally limited by the poor quality of the cells, such as cellular senescence and age-related functional decline [237, 238]. Genetic modification is thought to be an effective approach to reduce these limitations imposed on adult stem cells.

Some genetic disorders could be also treated with genetically modified stem cells. Human MSCs transfected with dystrophin could complement Duchenne muscular dystrophy myotubes via cellular fusion [239]. Chamberlain B.R. et al. disrupted dominant-negative mutant *COL1A1* collagen genes in MSCs from osteogenesis imperfecta patients, demonstrating successful gene targeting in adult human stem cells [240]. Other utilizations of gene delivery in stem cell research include the genetic labeling of the cells for *in vitro* or *in vivo* tracking. GFP is one of the most commonly used labeling genes which provides the convenience to study stem cell fate.

In our study, aimed on the improvement of understanding in polycation mediated gene delivery into adult stem cells, we used 25kDa B-PEI to deliver genes into bone

marrow derived human MSCs [Attached article 1]. The MSCs were donated by patients aged from 41 to 85 years old suffering cardiovascular disease. The gene delivery conditions were optimized in term of nitrogen/phosphorus ratio (N/P ratio) of PEI/DNA, PEI/DNA complex size and surface charge, DNA dosage, cell viability and transfection efficiency. The highest transfection efficiency was achieved at N/P ratio 2 and 6.0μg DNA/cm^2 culture area, while the cell viability under this condition was still at a high level (near 60%). We didn't observe the influence of age and gender of the patients on the transfection efficiency. The average transfection efficiency for cells of totality, middle-age group (donor age<65y), old-age group (donor age>65y), female group and male group was 4.32%, 3.85%, 4.52%, 4.14% and 4.38% respectively, as evaluated by flow cytometry. Interestingly, two subpopulations in the donors were observed; and in each, the transfection efficiency was linearly correlated to the cell percentage in S-phase. However, phenotypic characterization based on stem cell markers (CD29, CD44, CD45, CD73 and CD105) showed no significant differences between these two subpopulations. Finally, the transfer of therapeutic gene was studied using human VEGF165 plasmid. Result indicated that VEGF expression could be significantly enhanced by PEI to a clinical meaningful level.

Gene delivery could be also used for stem cells recruitment, in which stem cells are not transfected but attracted by signaling proteins (e.g. cytokine) expressed by other transfected cells. Although various tissue intrinsic stem cells have the capabilities of maintaining, generating and replacing, the limited stem cell pools are not sufficient to repair and regenerate damaged tissues [241]. Local delivery of chemotactic factors to recruit the stem cells from other tissues has been thought to be a promising therapeutic strategy to overcome this limitation in tissue regeneration [242]. In our study, plasmid DNA encoding stromal cell-derived factor-1α (SDF-1α) was delivered into cells via GAS mediated non-viral method [Attached article 4&5]. The expression of SDF-1α induced $CD117^+$ migration and homing both *in vitro* and *in vivo*. SDF-1α is a pivotal chemokine being able to guide stem cells to damaged tissues or organs [243]. Tissue repairs and functional improvements have been observed through SDF-1α mediated stem cells homing in many studies [244-250]. However, SDF-1α has very short half-life which is less then 15 minutes. It could be inactivated and cleaved by matrix metalloproteinase-2 (MMP-2) and CD26/dipeptidyl peptidase IV, which are abundant under inflammatory conditions [251-253]. *In situ* delivery of DNA encoding SDF-1α could lead to sustained

SDF-1α expression, and thereby improve the efficacy to recruit stem cells to the injured sites.

Results

1. PEI mediated genetic modification of human bone marrow MSCs

Genetic modification of stem cells is an effective approach to improve the efficacy of stem cell-based therapy. In our study, PEI mediated gene delivery into human bone marrow MSCs from patients was investigated [Attached article 1].

At N/P ratio 2 and 6.0μg DNA/cm^2 culture area, MSCs showed optimal transfection efficiency and high level viability (near 60%). The age and gender of the patients did not influence gene transfer efficiency. The average transfection efficiency of all samples, middle-age group (donor age<65y), old-age group (donor age>65y), female group and male group was 4.32%, 3.85%, 4.52%, 4.14% and 4.38% respectively. There was no significant difference between middle-age and old-age groups, as well as female and male groups. Of note, the transfection efficiency showed big variation among different individuals.

Interestingly, two subpopulations in the donors were observed; and in each, the transfection efficiency was linearly correlated to the cell percentage in S-phase. However, phenotypic characterization based on stem cell markers (CD29, CD44, CD45, CD73 and CD105) indicated that the cells of these two subpopulations were not significantly different.

The delivery of human VEGF165 gene by PEI led to a clinical meaningful level of transgene expression (3.49±0.52 pg VEGF/μg total protein), which was significantly higher than that of untransfected cells (1.84±0.11 pg VEGF/μg total protein) and naked DNA transfected cells (1.94±0.11 pg VEGF/μg total protein).

In this study, we investigated for the first time the influence of age and gender of donors on the gene transfer efficiency of human bone marrow MSCs mediated by PEI. We noticed a big variation of transfection efficiency among different individuals. By analyzing the data from multiple patients, we found two subpopulations in the donors according to the relationship between transgene expression efficiency and cell percentage in S-phase. However, the mechanism for the different behavior of these two subpopulations is not clear at present and needs to be further investigated. Finally, our result indicated that the expression of therapeutic gene VEGF could be enhanced by PEI

to a clinical meaningful level. In summary, this study improved our understanding of cationic polymer mediated gene delivery into human MSCs, and demonstrated the feasibility to use polymer for genetic modification of stem cells. Nevertheless, further study is still necessary to improve the gene delivery performance of polymer and clarity some cellular mechanism.

2. Recruitment of stem cells for cardiac function improvement

Matrigel is an injectable gelatinous mixture containing ECM components and various growth factors. In our study, matrigel was injected intracardiacly after MI in rat model, and the restoration of cardiac functions was assessed [Attached article 2].

The intracardiac administration of matrigel could enhance contraction kinetics of left ventricle. Compared with MI-PBS (myocardial infarcted, treated with PBS) group, MI-M (myocardial infarcted, treated with matrigel) hearts showed 22.7% increase in left ventricular ejection fraction (LV-EF), significantly enhanced peak rate of LV pressure rise and 24.5% increase in peak rate of LV pressure decline.

The intracardiac delivery of matrigel did not reduce infarction size. The infarction size of MI-M group (20.98±1.25%) showed no significant reduction compared with MI-PBS group (21.48±1.49%) 4 weeks after MI. However, the left ventricle wall thickness (LWT) of MI-M group (0.72±0.02mm) was significantly higher than that of MI-PBS group (0.62±0.02mm), indicating that matrigel could attenuate the decrease of infarct wall thickness. Moreover, MI-M hearts presented significantly higher capillary density in infarct border zone (130.88±4.7 vessels per HPF) compared with MI-PBS hearts (115.40±6.0 vessels per HPF), suggesting that matrigel could promote neoangiogenesis.

The local injection of matrigel could improve the recruitment of stem cells to the infarcted hearts. The number of $CD34^+$ and $CD117^+$ stem cells in MI-M hearts (13.0±1.51 $CD34^+$, 38.3±5.3 $CD117^+$ per HPF) was significantly higher than that in MI-PBS hearts (5.6±0.67 $CD34^+$, 25.7±1.5 $CD117^+$ per HPF) 4 weeks after MI.

In brief, we presented for the first time that intracardiac administration of matrigel after MI could increase the local number of $CD34^+$ and $CD117^+$ stem cells. Meanwhile, we provided the evidence that the recruited stem cells might promote the cardiac regeneration. Despite these encouraging findings, the exact mechanism by which matrigel act on stem cells and restore myocardial functions, has not been clearly

identified. We supposed that several potential factors might mediate this process. However, further study is necessary to confirm or clarify this mechanism.

3. Non-viral delivery of ASO for tumor inhibition

Differential mRNA splicing and alternative promoter usage of the *TP73* gene lead to the expression of multiple N-terminally truncated isoforms (ΔEx2, ΔEx2/3, ΔN', ΔN) that act as oncogenes. In our study, the delivery of LNA-ASO with non-viral method was performed to suppress tumor cell growth [Attached article 3].

ASO-116, which binds to ΔEx2/3p73 mRNA, was complexed by PEI. *In vitro*, PEI/ASO-116 polyplexes led to 6- to 9-fold decrease of target mRNA level on SK-Mel-29 cells after transfection. Due to this inhibitory effect on ΔEx2/3p73, PEI/ASO-116 treated cells showed significantly reduced proliferation rate over 5 days, compared with PEI/ASO-sc (scrambled control) treated cells and untreated cells.

In vivo, the distribution of PEI/ASO-116 polyplexes in the malignant melanoma tumors was investigated using fluorescence-labeled polyplexes. Results indicated that the polyplexes distributed within the whole tumor in 1 hour after intratumoral injection. Although the concentration of the polyplexes decreased over time in the tumor, a fraction still remained detectable after 24 hours, which is sufficient to allow continuous availability of polyplexes under daily administration.

In order to enhance the antitumoral efficacy, PEI/ASO-116 polyplexes were conjugated onto magnetic nanobeads (MNBs) and the MNB/PEI/ASO-116 complexes were intratumorally injected in the presence of a magnet implanted near the tumor. Magnetic force-restriction could prevent diffusion of the ASO from the injection site. As a result, MNB/PEI/ASO-116 complexes significantly reduced the tumor growth rate compared with PEI/ASO-116 polyplexes, as indicated by tumor growth curves. MNB/PEI/ASO-116 complexes and PEI/ASO-116 polyplexes offered an equally strong suppression of ΔEx2/3p73 expression (7-fold and 8.5-fold) compared with control group. However, MNB/PEI/ASO-116 complexes induced a more than 2 times higher increase of tumor suppressive TAp73. This indicates that enhanced specific therapeutic efficacy can be achieved by keeping the ASO concentrated in the tumor via magnetic force-restriction.

In summary, our results demonstrated that polymer mediated ASO delivery might be utilized for tumor inhibition. Coupled with inorganic MNBs, polymer can improve the *in vivo* administration of ASO and accordingly enhance the antitumoral efficacy. The data support the utilization of non-viral gene delivery method for cancer treatments.

4. GAS mediated gene delivery for stem cell recruitment

Gene activated matrixes have been used effectively in various applications due to their capability to allow local and sustained gene release to the desired site. In our study, gene activated substrates (GASs) were prepared by mixing PEI/DNA polyplexes with substrate materials (human fibronectin or rat tail collagen). The performance of the GASs on gene delivery and stem cell recruitment was investigated [Attached article 4&5].

GASs could allow sustained gene release over 2 months. On the first day, about 37% of total polyplexes was released from gene activated collagen (GAC). After that, the release speed slowed down and the cumulative amount of released polyplexes was approximately 41% after 67 days. Gene activated human fibronectin (GAH) presented more gently gene release. About 3% of polyplexes was released on the first day and up to 12% of polyplexes was released in 67 days.

GAC allowed high transfection efficiency and low cytotoxicity. At N/P ratio 4 and DNA dosage 10.0μg/cm^2, the transfection efficiency was 5.8×10^5 RLU/mg protein, and the cell viability was around 75% which was 1.85 times higher than that of substrate-free control group. GAH could also offer high transfection efficiency (5.6×10^6 RLU/mg protein at N/P ratio 4 and DNA dosage 7.5μg/cm^2), but no improvement of cell viability was observed.

Both GAC and GAH could be used for controllable gene transfer in designed area. Improtantly, the transfected cells could be used for stem cell recruitment. *In vitro*, rat MSCs transfected by SDF-1α-GAH and African green monkey kidney (COS7) cells transfected by SDF-1α-GAC showed the capability to guide the migration and homing of CD117$^+$ stem cells. *In vivo*, the implantation of SDF-1α-GAC into mouse hindlimb led to transgene express and consequent CD117$^+$ stem cells homing, whereas the induced inflammation significantly diminished in 2 weeks.

In this study, we prepared GASs to provide the homing signals that promote stem cells migration and recruitment. The GASs hold the advantage of allowing localized

gene delivery, sustained gene release, high transfection efficiency and low cytotoxicity. They can easily be coated onto the surface of scaffold for implantation. Our GASs containing SDF-1α gene induced $CD117^+$ stem cells migration and homing both *in vitro* and *in vivo*, showing the potential to overcome the limitation of low stem cell amount in intrinsic tissue pools for tissue repair. Furthermore, the sustained long-term SDF-1α gene expression by GASs might conquer the drawbacks associated with the direct administration of SDF-1α protein, since SDF-1α has very short half-life and can be inactivated and cleaved *in vivo*. In summary, the GASs provide a useful tool for stem cell based tissue engineering. They can also be used as model systems to study the molecular interplay between other adhesion molecules involved in stem cell therapy.

Conclusions

In present dissertation, PEI mediated non-viral gene delivery was studied and combined with stem cell-based therapy. We transfected human MSCs with PEI mediated gene delivery to study the genetic modification of adult stem cells [Attached article 1]. We evaluated the therapeutic effect of stem cells on restoration of heart functions after myocardial infarction in rat model [Attached article 2]. We delivered antisense oligonucleotide (ASO) with non-viral gene transfer method to inhibit tumor growth [Attached article 3]. And we developed a novel gene transfer technique called gene activated substrate (GAS) which might be used for stem cell recruitment [Attached article 4&5]. Based on our experimental results, some conclusions can be drawn as follows:

1. Human bone marrow derived MSCs could be genetically modified via PEI mediated gene delivery. The highest transfection efficiency was achieved at N/P ratio 2 and 6.0μg DNA/cm^2 culture area, while the cell viability under this condition was still at a high level. The donors' age and gender did not influence the gene transfer efficiency. Two subpopulations in the donors were observed; and in each, the transfection efficiency was linearly correlated to the cell percentage in S-phase. However, there were no phenotypic differences between these two subpopulations. The mechanism is still not clear at present and needs to be further studied. Finally, therapeutic gene expression was significantly enhanced by PEI onto a clinical meaningful level, suggesting the feasibility to use polymer for genetic modification of stem cells.
2. The recruitment of CD34$^+$ and CD117$^+$ stem cells might improve the restoration of heart functions after MI in rat model. After the delivery of Matrigel into myocardium by intracardiac injection following MI, the LV function, the infarct wall thickness of left ventricle and the capillary density of the hearts were significantly improved. The number of CD34$^+$ and CD117$^+$ stem cells was significantly increased in the Matrigel treated hearts. We assumed that the restoration of myocardial functions might attribute to the recruitment of stem cells.
3. Non-viral gene delivery could be applied to transfer not only DNA, but also other genetic materials. By delivering ASO with non-viral method, the tumor cell growth could be effectively inhibited both *in vitro* and *in vivo*. The

utilization of magnetic nanobeads (MNBs), onto which PEI/ASO polyplexes were conjugated, could improve the ASO transfer efficiency and thereby enhance the antitumoral efficacy.

4. Gene activated substrate (GAS) may allow localized gene delivery, sustained gene release, high transfection efficiency and low cytotoxicity. It could be easily coated onto scaffold for implantation. When SDF-1α gene was utilized, GAS could induce $CD117^{+}$ stem cell migration and homing both *in vitro* and *in vivo*. Thus, GAS shows high potential to recruit stem cells for regenerative therapy. As for the substrate materials, collagen was more suitable than fibronectin since collagen allowed higher cell viability.
5. Although presenting relative excellent performance for both *in vitro* and *in vivo* gene delivery, PEI showed some intrinsic drawbacks. The relative high cytotoxicity and non-biodegradability are the crucial ones. In order to achieve improved therapeutic effects, the transfection efficiency of PEI needs to be increased. Further improvements might focus on several directions. First is the chemical modification or conjugation to increase site-specificity, decrease cytotoxicity and enhance gene transfer efficiency. Second is the crosslinking of LMW-PEI via biodegradable bonds or polymers to provide biodegradability and reduce toxicity. Third is the synthesis of novel polymers that have the similar amines ratio (primary amine: secondary amine: tertiary amine) like PEI to allow high buffering capability.

In summary, this dissertation provides novel scientific-meaningful information regarding non-viral gene delivery and stem cell-based therapy. Further investigations in the fields of gene therapy and stem cell therapy need to be performed. It is senseful to consider the combination of these two therapeutic strategies, since it shows the potential to largely increase the therapeutic effects.

References

1. Segura T and Shea LD. Materials for non-viral gene delivery. *Annual Review of Materials Research*. 2001; 31: 25-46.
2. Thomas M and Klibanov AM. Non-viral gene therapy: polycation-mediated DNA delivery. *Applied Microbiology and Biotechnology*. 2003; 62 (1): 27-34.
3. Malone RW, Felgner PL, and Verma IM. Cationic Liposome-Mediated Rna Transfection. *Proceedings of the National Academy of Sciences of the United States of America*. 1989; 86 (16): 6077-6081.
4. Emmrich S, Wang WW, John K, Li WZ, and Putzer BM. Antisense gapmers selectively suppress individual oncogenic p73 splice isoforms and inhibit tumor growth in vivo. *Molecular Cancer*. 2009; 8: -.
5. Rauth S, Song KY, Ayares D, Wallace L, Moore PD, and Kucherlapati R. Transfection and Homologous Recombination Involving Single-Stranded-DNA Substrates in Mammalian-Cells and Nuclear Extracts. *Proceedings of the National Academy of Sciences of the United States of America*. 1986; 83 (15): 5587-5591.
6. Rich DP, Couture LA, Cardoza LM, Guiggio VM, Armentano D, Espino PC, Hehir K, Welsh MJ, Smith AE, and Gregory RJ. Development and Analysis of Recombinant Adenoviruses for Gene-Therapy of Cystic-Fibrosis. *Human Gene Therapy*. 1993; 4 (4): 461-476.
7. Asparuhova MB, Barde I, Trono D, Schranz K, and Schumperli D. Development and characterization of a triple combination gene therapy vector inhibiting HIV-1 multiplication. *Journal of Gene Medicine*. 2008; 10 (10): 1059-1070.
8. Ravet E, Lulka H, Gross F, Casteilla L, Buscail L, and Cordelier P. Using lentiviral vectors for efficient pancreatic cancer gene therapy. *Cancer Gene Therapy*. 2010; 17 (5): 315-324.
9. Cutroneo KR. Gene therapy for tissue regeneration. *Journal of Cellular Biochemistry*. 2003; 88 (2): 418-425.
10. Samson SL, Gonzalez EV, Yechoor V, Bajaj M, Oka K, and Chan L. Gene Therapy for Diabetes: Metabolic Effects of Helper-dependent Adenoviral Exendin 4 Expression in a Diet-induced Obesity Mouse Model (vol 16, pg 1805, 2008). *Molecular Therapy*. 2009; 17 (10): 1831-1831.
11. Li JR, Huang YW, Liang XY, Lu MJ, Li L, Yu L, and Deng RT. Plasmid DNA encoding antigens of infectious bursal disease viruses induce protective immune responses in chickens: factors influencing efficacy. *Virus Research*. 2003; 98 (1): 63-74.
12. Fannon MR. Gene expression in normal and disease states--identification of therapeutic targets. *Trends Biotechnol*. 1996; 14 (8): 294-8.
13. Macilwain C. World leaders heap praise on human genome landmark. *Nature*. 2000; 405: 983-4.
14. Anderson WF. Human gene therapy. *Nature*. 1998; 392 (6679): 25-30.
15. Boulaiz H, Marchal JA, Prados J, Melguizo C, and Aranega A. Non-viral and viral vectors for gene therapy. *Cellular and Molecular Biology*. 2005; 51 (1): 3-22.
16. Okumura K, Nakase M, Nakamura S, Kamei T, Inui M, and Tagawa T. Bax gene therapy for human osteosarcoma using cationic liposomes in vivo. *Oncology Reports*. 2007; 17 (4): 769-773.

17. Tanaka M and Grossman HB. In vivo gene therapy of human bladder cancer with PTEN suppresses tumor growth, downregulates phosphorylated Akt, and increases sensitivity to doxorubicin. *Gene Therapy*. 2003; 10 (19): 1636-1642.
18. Romano G, Michell P, Pacilio C, and Giordano A. Latest developments in gene transfer technology: achievements, perspectives, and controversies over therapeutic applications. *Stem Cells*. 2000; 18 (1): 19-39.
19. Lundstrom K and Boulikas T. Viral and non-viral vectors in gene therapy: Technology development and clinical trials. *Technology in Cancer Research & Treatment*. 2003; 2 (5): 471-485.
20. Chowdhury EH. Nuclear targeting of viral and non-viral DNA. *Expert Opin Drug Deliv*. 2009; 6 (7): 697-703.
21. Romano G, Pacilio C, and Giordano A. Gene transfer technology in therapy: current applications and future goals. *Stem Cells*. 1999; 17 (4): 191-202.
22. Zaiss AK and Muruve DA. Immune responses to adeno-associated virus vectors. *Current Gene Therapy*. 2005; 5 (3): 323-331.
23. Azzam T and Domb AJ. Current developments in gene transfection agents. *Curr Drug Deliv*. 2004; 1 (2): 165-93.
24. Sun JY, Anand-Jawa V, Chatterjee S, and Wong KK. Immune responses to adeno-associated virus and its recombinant vectors. *Gene Therapy*. 2003; 10 (11): 964-976.
25. Lavertu M, Methot S, Tran-Khanh N, and Buschmann MD. High efficiency gene transfer using chitosan/DNA nanoparticles with specific combinations of molecular weight and degree of deacetylation. *Biomaterials*. 2006; 27 (27): 4815-4824.
26. De Laporte L, Rea JC, and Shea LD. Design of modular non-viral gene therapy vectors. *Biomaterials*. 2006; 27 (7): 947-954.
27. Zeng JM, Wang X, and Wang S. Self-assembled ternary complexes of plasmid DNA, low molecular weight polyethylenimine and targeting peptide for nonviral gene delivery into neurons. *Biomaterials*. 2007; 28 (7): 1443-1451.
28. Aslan H, Zilberman Y, Arbeli V, Sheyn D, Matan Y, Liebergall M, Li JZ, Helm GA, Gazit D, and Gazit Z. Nucleofection-based ex vivo nonviral gene delivery to human stem cells as a platform for tissue regeneration. *Tissue Engineering*. 2006; 12 (4): 877-889.
29. Harrington KJ, Bateman AR, Melcher AA, Ahmed A, and Vile RG. Cancer gene therapy: Part 1. Vector development and regulation of gene expression. *Clinical Oncology*. 2002; 14 (1): 3-16.
30. Stopeck AT, Jones A, Hersh EM, Thompson JA, Finucane DM, Gutheil JC, and Gonzalez R. Phase II study of direct intralesional gene transfer of allovectin-7, an HLA-B7/beta 2-microglobulin DNA-liposome complex, in patients with metastatic melanoma. *Clinical Cancer Research*. 2001; 7 (8): 2285-2291.
31. Yoo GH, Hung MC, Lopez-Berestein G, LaFollette S, Ensley JF, Carey M, Batson E, Reynolds TC, and Murray JL. Phase I trial of intratumoral liposome E1A gene therapy in patients with recurrent breast and head and neck cancer. *Clinical Cancer Research*. 2001; 7 (5): 1237-1245.
32. Shah PB and Losordo DW. Non-Viral Vectors for Gene Therapy: Clinical Trials in Cardiovascular Disease. *Non-Viral Vectors for Gene Therapy, Second Edition: Part 2*. 2005; 54: 339-361.
33. Al-Dosari MS and Gao X. Nonviral Gene Delivery: Principle, Limitations, and Recent Progress. *Aaps Journal*. 2009; 11 (4): 671-681.

34. Villemejane J and Mir LM. Physical methods of nucleic acid transfer: general concepts and applications. *British Journal of Pharmacology*. 2009; 157 (2): 207-219.
35. Medina-Kauwe LK, Xie J, and Hamm-Alvarez S. Intracellular trafficking of nonviral vectors. *Gene Therapy*. 2005; 12 (24): 1734-1751.
36. Luzio JP, Mullock BM, Pryor PR, Lindsay MR, James DE, and Piper RC. Relationship between endosomes and lysosomes. *Biochemical Society Transactions*. 2001; 29: 476-480.
37. Li WJ, Nicol F, and Szoka FC. GALA: a designed synthetic pH-responsive amphipathic peptide with applications in drug and gene delivery. *Advanced Drug Delivery Reviews*. 2004; 56 (7): 967-985.
38. Xu YH and Szoka FC. Mechanism of DNA release from cationic liposome/DNA complexes used in cell transfection. *Biochemistry*. 1996; 35 (18): 5616-5623.
39. Boussif O, Lezoualch F, Zanta MA, Mergny MD, Scherman D, Demeneix B, and Behr JP. A Versatile Vector for Gene and Oligonucleotide Transfer into Cells in Culture and in-Vivo - Polyethylenimine. *Proceedings of the National Academy of Sciences of the United States of America*. 1995; 92 (16): 7297-7301.
40. Akinc A, Thomas M, Klibanov AM, and Langer R. Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis. *Journal of Gene Medicine*. 2005; 7 (5): 657-663.
41. Moret I, Peris JE, Guillem VM, Benet M, Revert F, Dasi F, Crespo A, and Alino SF. Stability of PEI-DNA and DOTAP-DNA complexes: effect of alkaline pH, heparin and serum. *Journal of Controlled Release*. 2001; 76 (1-2): 169-181.
42. Bachand M, Trent AM, Bunker BC, and Bachand GD. Physical factors affecting kinesin-based transport of synthetic nanoparticle cargo. *Journal of Nanoscience and Nanotechnology*. 2005; 5 (5): 718-722.
43. Bastos R, Pante N, and Burke B. Nuclear pore complex proteins. *Int Rev Cytol*. 1995; 162B: 257-302.
44. Wente SR. Gatekeepers of the nucleus. *Science*. 2000; 288 (5470): 1374-7.
45. Chan CK and Jans DA. Using nuclear targeting signals to enhance non-viral gene transfer. *Immunology and Cell Biology*. 2002; 80 (2): 119-130.
46. Cartier R and Reszka R. Utilization of synthetic peptides containing nuclear localization signals for nonviral gene transfer systems. *Gene Therapy*. 2002; 9 (3): 157-167.
47. Dean DA, Dean BS, Muller S, and Smith LC. Sequence requirements for plasmid nuclear import. *Experimental Cell Research*. 1999; 253 (2): 713-722.
48. Dean DA. Import of plasmid DNA into the nucleus is sequence specific. *Experimental Cell Research*. 1997; 230 (2): 293-302.
49. Vacik J, Dean BS, Zimmer WE, and Dean DA. Cell-specific nuclear import of plasmid DNA. *Gene Therapy*. 1999; 6 (6): 1006-1014.
50. Dean DA, Strong DD, and Zimmer WE. Nuclear entry of nonviral vectors. *Gene Therapy*. 2005; 12 (11): 881-890.
51. Brunner S, Sauer T, Carotta S, Cotten M, Saltik M, and Wagner E. Cell cycle dependence of gene transfer by lipoplex polyplex and recombinant adenovirus. *Gene Therapy*. 2000; 7 (5): 401-407.
52. Brunner S, Furtbauer E, Sauer T, Kursa M, and Wagner E. Overcoming the nuclear barrier: Cell cycle independent nonviral gene transfer with linear polyethylenimine or electroporation. *Molecular Therapy*. 2002; 5 (1): 80-86.

53. Mannisto M, Ronkko S, Matto M, Honkakoski P, Hyttinen M, Pelkonen J, and Urtti A. The role of cell cycle on polyplex-mediated gene transfer into a retinal pigment epithelial cell line. *Journal of Gene Medicine*. 2005; 7 (4): 466-476.
54. Prasmickaite L, Hogset A, and Berg K. The role of the cell cycle on the efficiency of photochemical gene transfection. *Biochimica Et Biophysica Acta-General Subjects*. 2002; 1570 (3): 210-218.
55. Bieber T, Meissner W, Kostin S, Niemann A, and Elsasser HP. Intracellular route and transcriptional competence of polyethylenimine-DNA complexes. *Journal of Controlled Release*. 2002; 82 (2-3): 441-454.
56. Godbey WT, Wu KK, and Mikos AG. Tracking the intracellular path of poly(ethylenimine)/DNA complexes for gene delivery. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96 (9): 5177-5181.
57. Godbey WT, Barry MA, Saggau P, Wu KK, and Mikos AG. Poly(ethylenimine)-mediated transfection: A new paradigm for gene delivery. *Journal of Biomedical Materials Research*. 2000; 51 (3): 321-328.
58. Davis HL, Whalen RG, and Demeneix BA. Direct Gene-Transfer into Skeletal-Muscle Invivo - Factors Affecting Efficiency of Transfer and Stability of Expression. *Human Gene Therapy*. 1993; 4 (2): 151-159.
59. Wolf DP, Thomson JA, Zelinskiwooten MB, and Stouffer RL. Invitro Fertilization-Embryo Transfer in Nonhuman-Primates - the Technique and Its Applications. *Molecular Reproduction and Development*. 1990; 27 (3): 261-280.
60. Barber MA. A technic for the inoculation of bacteria and other substances into living cells. *Journal of Infectious Diseases*. 1911; 8 (3): 348-360.
61. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, and Felgner PL. Direct Gene-Transfer into Mouse Muscle Invivo. *Science*. 1990; 247 (4949): 1465-1468.
62. Herweijer H and Wolff JA. Progress and prospects: naked DNA gene transfer and therapy. *Gene Therapy*. 2003; 10 (6): 453-458.
63. Lui VWY, Falo LD, and Huang L. Systemic production of IL-12 by naked DNA mediated gene transfer: toxicity and attenuation of transgene expression in vivo. *Journal of Gene Medicine*. 2001; 3 (4): 384-393.
64. Sato Y, Yamauchi N, Takahashi M, Sasaki K, Fukaura J, Neda H, Fujii S, Hirayama M, Itoh Y, Koshita Y, Kogawa K, Kato J, Sakamaki S, and Niitsu Y. In vivo gene delivery to tumor cells by transferrin-streptavidin-DNA conjugate. *Faseb Journal*. 2000; 14 (13): 2108-2118.
65. Desigaux L, Gourden C, Roufai MB, Richard P, Oudrhiri N, Lehn P, Escande D, Pollard H, and Pitard B. Nonionic amphiphilic block copolymers promote gene transfer to the lung. *Human Gene Therapy*. 2005; 16 (7): 821-829.
66. Freeman DJ and Niven RW. The influence of sodium glycocholate and other additives on the in vivo transfection of plasmid DNA in the lungs. *Pharmaceutical Research*. 1996; 13 (2): 202-209.
67. Glasspool-Malone J and Malone RW. Marked enhancement of direct respiratory tissue transfection by aurintricarboxylic acid. *Human Gene Therapy*. 1999; 10 (10): 1703-1713.
68. Schreurs MWJ, de Boer AJ, Figdor CG, and Adema GJ. Genetic vaccination against the melanocyte lineage-specific antigen gp100 induces cytotoxic T lymphocyte-mediated tumor protection. *Cancer Research*. 1998; 58 (12): 2509-2514.

69. Schultz J, Pavlovic J, Strack B, Nawrath M, and Moelling K. Long-lasting anti-metastatic efficiency of interleukin 12-encoding plasmid DNA. *Human Gene Therapy*. 1999; 10 (3): 407-417.
70. Song K, Chang Y, and Prud'homme GJ. Regulation of T-helper-1 versus T-helper-2 activity and enhancement of tumor immunity by combined DNA-based vaccination and nonviral cytokine gene transfer. *Gene Therapy*. 2000; 7 (6): 481-492.
71. Losordo DW, Vale PR, Hendel RC, Milliken CE, Fortuin FD, Cummings N, Schatz RA, Asahara T, Isner JM, and Kuntz RE. Phase 1/2 placebo-controlled, double-blind, dose-escalating trial of myocardial vascular endothelial growth factor 2 gene transfer by catheter delivery in patients with chronic myocardial ischemia. *Circulation*. 2002; 105 (17): 2012-2018.
72. Wendell DM, Hemond BD, Hogan NC, Taberner AJ, and Hunter IW. The effect of jet parameters on jet injection. *Conf Proc IEEE Eng Med Biol Soc*. 2006; 1: 5005-8.
73. Ren S, Li M, Smith JM, DeTolla LJ, and Furth PA. Low-volume jet injection for intradermal immunization in rabbits. *BMC Biotechnol*. 2002; 2: 10.
74. Stein U, Walther W, Stege A, Kaszubiak A, Fichtner I, and Lage H. Complete in vivo reversal of the multidrug resistance phenotype by jet-injection of anti-MDR1 short hairpin RNA-encoding plasmid DNA. *Molecular Therapy*. 2008; 16 (1): 178-86.
75. Walther W, Siegel R, Kobelt D, Knosel T, Dietel M, Bembenek A, Aumann J, Schleef M, Baier R, Stein U, and Schlag PM. Novel jet-injection technology for nonviral intratumoral gene transfer in patients with melanoma and breast cancer. *Clinical Cancer Research*. 2008; 14 (22): 7545-53.
76. Lysakowski C, Dumont L, Tramer MR, and Tassonyi E. A needle-free jet-injection system with lidocaine for peripheral intravenous cannula insertion: a randomized controlled trial with cost-effectiveness analysis. *Anesth Analg*. 2003; 96 (1): 215-9, table of contents.
77. Klein TM, Wolf ED, Wu R, and Sanford JC. High-Velocity Microprojectiles for Delivering Nucleic-Acids into Living Cells. *Nature*. 1987; 327 (6117): 70-73.
78. Yang NS, Burkholder J, Roberts B, Martinell B, and McCabe D. In vivo and in vitro gene transfer to mammalian somatic cells by particle bombardment. *Proc Natl Acad Sci U S A*. 1990; 87 (24): 9568-72.
79. Gao X, Kim KS, and Liu DX. Nonviral gene delivery: What we know and what is next. *Aaps Journal*. 2007; 9 (1): E92-E104.
80. Uchida M, Natsume H, Kobayashi D, Sugibayashi K, and Morimoto Y. Effects of particle size, helium gas pressure and microparticle dose on the plasma concentration of indomethacin after bombardment of indomethacin-loaded poly-L-lactic acid microspheres using a Helios (TM) gun system. *Biological & Pharmaceutical Bulletin*. 2002; 25 (5): 690-693.
81. Neumann E, Schaeferridder M, Wang Y, and Hofschneider PH. Gene-Transfer into Mouse Lyoma Cells by Electroporation in High Electric-Fields. *Embo Journal*. 1982; 1 (7): 841-845.
82. Titomirov AV, Sukharev S, and Kistanova E. In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. *Biochim Biophys Acta*. 1991; 1088 (1): 131-4.
83. Canatella PJ and Prausnitz MR. Prediction and optimization of gene transfection and drug delivery by electroporation. *Gene Therapy*. 2001; 8 (19): 1464-9.

84. Heller LC, Ugen K, and Heller R. Electroporation for targeted gene transfer. *Expert Opin Drug Deliv*. 2005; 2 (2): 255-68.
85. Hasson E, Slovatizky Y, Shimoni Y, Falk H, Panet A, and Mitrani E. Solid tissues can be manipulated ex vivo and used as vehicles for gene therapy. *Journal of Gene Medicine*. 2005; 7 (7): 926-935.
86. Dean DA, Machado-Aranda D, Blair-Parks K, Yeldandi AV, and Young JL. Electroporation as a method for high-level nonviral gene transfer to the lung. *Gene Therapy*. 2003; 10 (18): 1608-1615.
87. Magin-Lachmann C, Kotzamanis G, D'Aiuto L, Cooke H, Huxley C, and Wagner E. In vitro and in vivo delivery of intact BAC DNA - comparison of different methods. *Journal of Gene Medicine*. 2004; 6 (2): 195-209.
88. Molnar MJ, Gilbert R, Lu YF, Liu AB, Guo A, Larochelle N, Orlopp K, Lochmuller H, Petrof BJ, Nalbantoglu J, and Karpati G. Factors influencing the efficacy, longevity, and safety of electroporation-assisted plasmid-based gene transfer into mouse muscles. *Molecular Therapy*. 2004; 10 (3): 447-455.
89. Marti G, Ferguson M, Wang J, Byrnes C, Dieb R, Qaiser R, Bonde P, Duncan MD, and Harmon JW. Electroporative transfection with KGF-1 DNA improves wound healing in a diabetic mouse model. *Gene Therapy*. 2004; 11 (24): 1780-1785.
90. Sakai M, Nishikawa M, Thanaketpaisarn O, Yamashita F, and Hashida M. Hepatocyte-targeted gene transfer by combination of vascularly delivered plasmid DNA and in vivo electroporation. *Gene Therapy*. 2005; 12 (7): 607-616.
91. McMahon JM and Wells DJ. Electroporation for gene transfer to skeletal muscles - Current status. *Biodrugs*. 2004; 18 (3): 155-165.
92. McMahon JM, Signori E, Wells KE, Fazio VM, and Wells DJ. Optimisation of electrotransfer of plasmid into skeletal muscle by pretreatment with hyaluronidase - increased expression with reduced muscle damage. *Gene Therapy*. 2001; 8 (16): 1264-1270.
93. Andre F and Mir LM. DNA electrotransfer: its principles and an updated review of its therapeutic applications. *Gene Therapy*. 2004; 11: S33-S42.
94. Durieux AC, Bonnefoy R, Busso T, and Freyssenet D. In vivo gene electrotransfer into skeletal muscle: effects of plasmid DNA on the occurrence and extent of muscle damage. *Journal of Gene Medicine*. 2004; 6 (7): 809-816.
95. Gissel H and Clausen T. Excitation-induced Ca2+ influx and skeletal muscle cell damage. *Acta Physiologica Scandinavica*. 2001; 171 (3): 327-334.
96. ter Haar G. Therapeutic applications of ultrasound. *Progress in Biophysics & Molecular Biology*. 2007; 93 (1-3): 111-129.
97. Kim HJ, Greenleaf JF, Kinnick RR, Bronk JT, and Bolander ME. Ultrasound-mediated transfection of mammalian cells. *Human Gene Therapy*. 1996; 7 (11): 1339-1346.
98. Liang HD, Lu QL, Xue SA, Halliwell M, Kodama T, Cosgrove DO, Stauss HJ, Partridge TA, and Blomley MJ. Optimisation of ultrasound-mediated gene transfer (sonoporation) in skeletal muscle cells. *Ultrasound in Medicine and Biology*. 2004; 30 (11): 1523-9.
99. Endoh M, Koibuchi N, Sato M, Morishita R, Kanzaki T, Murata Y, and Kaneda Y. Fetal gene transfer by intrauterine injection with microbubble-enhanced ultrasound. *Molecular Therapy*. 2002; 5 (5): 501-508.
100. Nozaki T, Ogawa R, Feril LB, Kagiya G, Fuse H, and Kondo T. Enhancement of ultrasound-mediated gene transfection by membrane modification. *Journal of Gene Medicine*. 2003; 5 (12): 1046-1055.

101. Ogawa R, Kagiya G, Feril LB, Nakaya N, Nozaki T, Fuse H, and Kondo T. Ultrasound mediated intravesical transfection enhanced by treatment with lidocaine or heat. *Journal of Urology*. 2004; 172 (4): 1469-1473.
102. Koch S, Pohl P, Cobet U, and Rainov NG. Ultrasound enhancement of liposome-mediated cell transfection is caused by cavitation effects. *Ultrasound in Medicine and Biology*. 2000; 26 (5): 897-903.
103. Anwer K, Kao G, Proctor B, Anscombe I, Florack V, Earls R, Wilson E, McCreery T, Unger E, Rolland A, and Sullivan SM. Ultrasound enhancement of cationic lipid-mediated gene transfer to primary tumors following systemic administration. *Gene Therapy*. 2000; 7 (21): 1833-1839.
104. Tsunoda S, Mazda O, Oda Y, Iida Y, Akabame S, Kishida T, Masaharu SY, Asada H, Gojo S, Imanishi J, Matsubara H, and Yoshikawa T. Sonoporation using microbubble BR14 promotes pDNA/siRNA transduction to murine heart. *Biochemical and Biophysical Research Communications*. 2005; 336 (1): 118-127.
105. Unger EC, Hersh E, Vannan M, Matsunaga TO, and McCreery M. Local drug and gene delivery through microbubbles. *Progress in Cardiovascular Diseases*. 2001; 44 (1): 45-54.
106. Sheyn D, Kimelman-Bleich N, Pelled G, Zilberman Y, Gazit D, and Gazit Z. Ultrasound-based nonviral gene delivery induces bone formation in vivo. *Gene Therapy*. 2008; 15 (4): 257-266.
107. Taniyama Y, Tachibana K, Hiraoka K, Aoki M, Yamamoto S, Matsumoto K, Nakamura T, Ogihara T, Kaneda Y, and Morishita R. Development of safe and efficient novel nonviral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. *Gene Therapy*. 2002; 9 (6): 372-380.
108. Sheikov N, McDannold N, Sharma S, and Hynynen K. Effect of focused ultrasound applied with an ultrasound contrast agent on the tight junctional integrity of the brain microvascular endothelium. *Ultrasound in Medicine and Biology*. 2008; 34 (7): 1093-1104.
109. Zhang G, Song YK, and Liu D. Long-term expression of human alpha1-antitrypsin gene in mouse liver achieved by intravenous administration of plasmid DNA using a hydrodynamics-based procedure. *Gene Therapy*. 2000; 7 (15): 1344-9.
110. Liu F, Song YK, and Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. *Gene Therapy*. 1999; 6 (7): 1258-1266.
111. Zhang G, Gao X, Song YK, Vollmer R, Stolz DB, Gasiorowski JZ, Dean DA, and Liu D. Hydroporation as the mechanism of hydrodynamic delivery. *Gene Therapy*. 2004; 11 (8): 675-682.
112. Miao CH, Thompson AR, Loeb K, and Ye X. Long-term and therapeutic-level hepatic gene expression of human factor IX after naked plasmid transfer in vivo. *Molecular Therapy*. 2001; 3 (6): 947-57.
113. Miao CH, Ye X, and Thompson AR. High-level factor VIII gene expression in vivo achieved by nonviral liver-specific gene therapy vectors. *Human Gene Therapy*. 2003; 14 (14): 1297-1305.
114. Alino SF, Crespo A, and Dasi F. Long-term therapeutic levels of human alpha-1 antitrypsin in plasma after hydrodynamic injection of nonviral DNA. *Gene Therapy*. 2003; 10 (19): 1672-9.

115. Stoll SM, Sclimenti CR, Baba EJ, Meuse L, Kay MA, and Calos MP. Epstein-Barr virus/human vector provides high-level, long-term expression of alpha1-antitrypsin in mice. *Molecular Therapy*. 2001; 4 (2): 122-9.
116. Jiang J, Yamato E, and Miyazaki J. Intravenous delivery of naked plasmid DNA for in vivo cytokine expression. *Biochem Biophys Res Commun*. 2001; 289 (5): 1088-92.
117. Yang J, Chen S, Huang L, Michalopoulos GK, and Liu Y. Sustained expression of naked plasmid DNA encoding hepatocyte growth factor in mice promotes liver and overall body growth. *Hepatology*. 2001; 33 (4): 848-59.
118. Maruyama H, Higuchi N, Kameda S, Miyazaki J, and Gejyo F. Rat liver-targeted naked plasmid DNA transfer by tail vein injection. *Mol Biotechnol*. 2004; 26 (2): 165-72.
119. Al-Dosari MS, Knapp JE, and Liu D. Hydrodynamic delivery. *Adv Genet*. 2005; 54: 65-82.
120. Fabre JW, Grehan A, Whitehorne M, Sawyer GJ, Dong X, Salehi S, Eckley L, Zhang X, Seddon M, Shah AM, Davenport M, and Rela M. Hydrodynamic gene delivery to the pig liver via an isolated segment of the inferior vena cava. *Gene Therapy*. 2008; 15 (6): 452-462.
121. Alino SF, Herrero MJ, Noguera I, Dasi F, and Sanchez M. Pig liver gene therapy by noninvasive interventionist catheterism. *Gene Therapy*. 2007; 14 (4): 334-343.
122. Yoshino H, Hashizume K, and Kobayashi E. Naked plasmid DNA transfer to the porcine liver using rapid injection with large volume. *Gene Therapy*. 2006; 13 (24): 1696-1702.
123. Suda T, Suda K, and Liu DX. Computer-assisted hydrodynamic gene delivery. *Molecular Therapy*. 2008; 16 (6): 1098-1104.
124. Liu F and Huang L. Noninvasive gene delivery to the liver by mechanical massage. *Hepatology*. 2002; 35 (6): 1314-1319.
125. Liu F, Lei J, Vollmer R, and Huang L. Mechanism of liver gene transfer by mechanical massage. *Molecular Therapy*. 2004; 9 (3): 452-457.
126. Schatzlein AG. Non-viral vectors in cancer gene therapy: principles and progress. *Anti-Cancer Drugs*. 2001; 12 (4): 275-304.
127. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM, and Danielsen M. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci U S A*. 1987; 84 (21): 7413-7.
128. Hashida M, Kawakami S, and Yamashita F. Lipid carrier systems for targeted drug and gene delivery. *Chem Pharm Bull (Tokyo)*. 2005; 53 (8): 871-80.
129. Zuhorn IS, Engberts JB, and Hoekstra D. Gene delivery by cationic lipid vectors: overcoming cellular barriers. *Eur Biophys J*. 2007; 36 (4-5): 349-62.
130. Pedroso de Lima MC, Simoes S, Pires P, Faneca H, and Duzgunes N. Cationic lipid-DNA complexes in gene delivery: from biophysics to biological applications. *Adv Drug Deliv Rev*. 2001; 47 (2-3): 277-94.
131. Liu D, Ren T, and Gao X. Cationic transfection lipids. *Curr Med Chem*. 2003; 10 (14): 1307-15.
132. Chesnoy S and Huang L. Structure and function of lipid-DNA complexes for gene delivery. *Annu Rev Biophys Biomol Struct*. 2000; 29: 27-47.
133. Chesnoy S and Huang L. Structure and function of lipid-DNA complexes for gene delivery. *Annual Review of Biophysics and Biomolecular Structure*. 2000; 29: 27-47.

134. Pinnaduwage P, Schmitt L, and Huang L. Use of a Quaternary Ammonium Detergent in Liposome Mediated DNA Transfection of Mouse L-Cells. *Biochimica Et Biophysica Acta*. 1989; 985 (1): 33-37.
135. Porteous DJ, Dorin JR, McLachlan G, DavidsonSmith H, Davidson H, Stevenson BJ, Carothers AD, Wallace WAH, Moralee S, Hoenes C, Kallmeyer G, Michaelis U, Naujoks K, Ho LP, Samways JM, Imrie M, Greening AP, and Innes JA. Evidence for safety and efficacy of DOTAP cationic liposome mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Gene Therapy*. 1997; 4 (3): 210-218.
136. Vigneron JP, Oudrhiri N, Fauquet M, Vergely L, Bradley JC, Basseville M, Lehn P, and Lehn JM. Guanidinium-cholesterol cationic lipids: Efficient vectors for the transfection of eukaryotic cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93 (18): 9682-9686.
137. Ren T, Zhang GS, Liu F, and Liu DX. Synthesis and evaluation of vitamin D-based cationic lipids for gene delivery in vitro. *Bioorganic & Medicinal Chemistry Letters*. 2000; 10 (9): 891-894.
138. Tang FX and Hughes JA. Introduction of a disulfide bond into a cationic lipid enhances transgene expression of plasmid DNA. *Biochemical and Biophysical Research Communications*. 1998; 242 (1): 141-145.
139. Liu F, Qi H, Huang L, and Liu D. Factors controlling the efficiency of cationic lipid-mediated transfection in vivo via intravenous administration. *Gene Therapy*. 1997; 4 (6): 517-523.
140. Hui SW, Langner M, Zhao YL, Ross P, Hurley E, and Chan K. The role of helper lipids in cationic liposome-mediated gene transfer. *Biophysical Journal*. 1996; 71 (2): 590-599.
141. Monk KWC and Cullis PR. Structural and fusogenic properties of cationic liposomes in the presence of plasmid DNA. *Biophysical Journal*. 1997; 73 (5): 2534-2545.
142. Simoes S, Slepushkin V, Gaspar R, de Lima MCP, and Duzgunes N. Gene delivery by negatively charged ternary complexes of DNA, cationic liposomes and transferrin or fusigenic peptides. *Gene Therapy*. 1998; 5 (7): 955-964.
143. Felgner JH, Kumar R, Sridhar CN, Wheeler CJ, Tsai YJ, Border R, Ramsey P, Martin M, and Felgner PL. Enhanced Gene Delivery and Mechanism Studies with a Novel Series of Cationic Lipid Formulations. *Journal of Biological Chemistry*. 1994; 269 (4): 2550-2561.
144. Koltover I, Salditt T, Radler JO, and Safinya CR. An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery. *Science*. 1998; 281 (5373): 78-81.
145. Zuidam NJ and Barenholz Y. Electrostatic and structural properties of complexes involving plasmid DNA and cationic lipids commonly used for gene delivery. *Biochimica Et Biophysica Acta-Biomembranes*. 1998; 1368 (1): 115-128.
146. Wheeler CJ, Felgner PL, Tsai YJ, Marshall J, Sukhu L, Doh SG, Hartikka J, Nietupski J, Manthorpe M, Nichols M, Plewe M, Liang X, Norman J, Smith A, and Cheng SH. A novel cationic lipid greatly enhances plasmid DNA delivery and expression in mouse lung. *Proc Natl Acad Sci U S A*. 1996; 93 (21): 11454-9.
147. Harvie P, Wong FMP, and Bally MB. Characterization of lipid DNA interactions. I. Destabilization of bound lipids and DNA dissociation. *Biophysical Journal*. 1998; 75 (2): 1040-1051.

148. Simoes S, Slepushkin V, Pires P, Gaspar R, de Lima MCP, and Duzgunes N. Mechanisms of gene transfer mediated by lipoplexes associated with targeting ligands or pH-sensitive peptides. *Gene Therapy*. 1999; 6 (11): 1798-1807.
149. Wang JK, Guo X, Xu YH, Barron L, and Szoka FC. Synthesis and characterization of long chain alkyl acyl carnitine esters. Potentially biodegradable cationic lipids for use in gene delivery. *Journal of Medicinal Chemistry*. 1998; 41 (13): 2207-2215.
150. Liu Y, Mounkes LC, Liggitt HD, Brown CS, Solodin I, Heath TD, and Debs RJ. Factors influencing the efficiency of cationic liposome-mediated intravenous gene delivery. *Nature Biotechnology*. 1997; 15 (2): 167-173.
151. Hong KL, Zheng WW, Baker A, and Papahadjopoulos D. Stabilization of cationic liposome-plasmid DNA complexes by polyamines and poly(ethylene glycol)-phospholipid conjugates for efficient in vivo gene delivery. *Febs Letters*. 1997; 400 (2): 233-237.
152. Song YK and Liu DX. Free liposomes enhance the transfection activity of DNA/lipid complexes in vivo by intravenous administration. *Biochimica Et Biophysica Acta-Biomembranes*. 1998; 1372 (1): 141-150.
153. Li S, Rizzo MA, Bhattacharya S, and Huang L. Characterization of cationic lipid-protamine-DNA (LPD) complexes for intravenous gene delivery. *Gene Therapy*. 1998; 5 (7): 930-937.
154. Sternberg B, Sorgi FL, and Huang L. New Structures in Complex-Formation between DNA and Cationic Liposomes Visualized by Freeze-Fracture Electron-Microscopy. *Febs Letters*. 1994; 356 (2-3): 361-366.
155. Radler JO, Koltover I, Salditt T, and Safinya CR. Structure of DNA-cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes. *Science*. 1997; 275 (5301): 810-814.
156. Templeton NS, Lasic DD, Frederik PM, Strey HH, Roberts DD, and Pavlakis GN. Improved DNA: Liposome complexes for increased systemic delivery and gene expression. *Nature Biotechnology*. 1997; 15 (7): 647-652.
157. Almofti MR, Harashima H, Shinohara Y, Almofti A, Li WH, and Kiwada H. Lipoplex size determines lipofection efficiency with or without serum. *Molecular Membrane Biology*. 2003; 20 (1): 35-43.
158. Ross PC and Hui SW. Lipoplex size is a major determinant of in vitro lipofection efficiency. *Gene Therapy*. 1999; 6 (4): 651-659.
159. Goncalves E, Debs RJ, and Heath TD. The effect of liposome size on the final lipid/DNA ratio of cationic lipoplexes. *Biophysical Journal*. 2004; 86 (3): 1554-1563.
160. Gershon H, Ghirlando R, Guttman SB, and Minsky A. Mode of Formation and Structural Features of DNA Cationic Liposome Complexes Used for Transfection. *Biochemistry*. 1993; 32 (28): 7143-7151.
161. Hirko A, Tang FX, and Hughes JA. Cationic lipid vectors for plasmid DNA delivery. *Current Medicinal Chemistry*. 2003; 10 (14): 1185-1193.
162. Ferrari ME, Rusalov D, Enas J, and Wheeler CJ. Synergy between cationic lipid and co-lipid determines the macroscopic structure and transfection activity of lipoplexes. *Nucleic Acids Research*. 2002; 30 (8): 1808-1816.
163. Congiu A, Pozzi D, Esposito C, Castellano C, and Mossa G. Correlation between structure and transfection efficiency: a study of DC-Chol-DOPE/DNA complexes. *Colloids and Surfaces B-Biointerfaces*. 2004; 36 (1): 43-48.
164. Liu Y, Fong S, and Debs RJ. Cationic liposome-mediated gene delivery in vivo. *Methods Enzymol*. 2003; 373: 536-50.

165. Elouahabi A and Ruysschaert JM. Formation and intracellular trafficking of lipoplexes and polyplexes. *Molecular Therapy*. 2005; 11 (3): 336-47.
166. Brunner S, Sauer T, Carotta S, Cotten M, Saltik M, and Wagner E. Cell cycle dependence of gene transfer by lipoplex, polyplex and recombinant adenovirus. *Gene Therapy*. 2000; 7 (5): 401-7.
167. Ishii T, Okahata Y, and Sato T. Mechanism of cell transfection with plasmid/chitosan complexes. *Biochimica Et Biophysica Acta-Biomembranes*. 2001; 1514 (1): 51-64.
168. Sato T, Ishii T, and Okahata Y. In vitro gene delivery mediated by chitosan. Effect of pH, serum, and molecular mass of chitosan on the transfection efficiency. *Biomaterials*. 2001; 22 (15): 2075-2080.
169. Tang MX, Redemann CT, and Szoka FC, Jr. In vitro gene delivery by degraded polyamidoamine dendrimers. *Bioconjug Chem*. 1996; 7 (6): 703-14.
170. Wu GY and Wu CH. Receptor-mediated in vitro gene transformation by a soluble DNA carrier system. *Journal of Biological Chemistry*. 1987; 262 (10): 4429-32.
171. Emi N, Kidoaki S, Yoshikawa K, and Saito H. Gene transfer mediated by polyarginine requires a formation of big carrier-complex of DNA aggregate. *Biochemical and Biophysical Research Communications*. 1997; 231 (2): 421-424.
172. Koo H, Jin GW, and Park JS. In vitro Gene Delivery to HepG2 Cells with a Novel Galactosylated Polyornithine. *Bulletin of the Korean Chemical Society*. 2009; 30 (7): 1622-1624.
173. Ramsay E and Gumbleton M. Polylysine and polyornithine gene transfer complexes: A study of complex stability and cellular uptake as a basis for their differential in-vitro transfection efficiency. *Journal of Drug Targeting*. 2002; 10 (1): 1-9.
174. Balicki D, Putnam CD, Scaria PV, and Beutler E. Structure and function correlation in histone H2A peptide-mediated gene transfer. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99 (11): 7467-7471.
175. Park YJ, Liang JF, Ko KS, Kim SW, and Yang VC. Low molecular weight protamine as an efficient and nontoxic gene carrier: in vitro study. *Journal of Gene Medicine*. 2003; 5 (8): 700-711.
176. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, and Behr JP. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc Natl Acad Sci U S A*. 1995; 92 (16): 7297-301.
177. Li W, Ma N, Ong LL, Nesselmann C, Klopsch C, Ladilov Y, Furlani D, Piechaczek C, Moebius JM, Lutzow K, Lendlein A, Stamm C, Li RK, and Steinhoff G. Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. *Stem Cells*. 2007; 25 (8): 2118-27.
178. Wang J, Mao HQ, and Leong KW. A novel biodegradable gene carrier based on polyphosphoester. *J Am Chem Soc*. 2001; 123 (38): 9480-1.
179. Marschall P, Malik N, and Larin Z. Transfer of YACs up to 2.3 Mb intact into human cells with polyethylenimine. *Gene Therapy*. 1999; 6 (9): 1634-1637.
180. Campeau P, Chapdelaine P, Seigneurin-Venin S, Massie B, and Tremblay JP. Transfection of large plasmids in primary human myoblasts. *Gene Therapy*. 2001; 8 (18): 1387-1394.

181. Fischer D, Bieber T, Li YX, Elsasser HP, and Kissel T. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: Effect of molecular weight on transfection efficiency and cytotoxicity. *Pharmaceutical Research*. 1999; 16 (8): 1273-1279.
182. Wang WW, Li WZ, Ong LL, Furlani D, Kaminski A, Liebold A, Lutzow K, Lendlein A, Wang J, Li RK, Steinhoff G, and Ma N. Localized SDF-1alpha gene release mediated by collagen substrate induces CD117+stem cells homing. *Journal of Cellular and Molecular Medicine*. 2010; 14 (1-2): 392-402.
183. Wang W, Li W, Ong LL, Lutzow K, Lendlein A, Furlani D, Gabel R, Kong D, Wang J, Li RK, Steinhoff G, and Ma N. Localized and sustained SDF-1 gene release mediated by fibronectin films: A potential method for recruiting stem cells. *Int J Artif Organs*. 2009; 32 (3): 141-9.
184. Goula D, Benoist C, Mantero S, Merlo G, Levi G, and Demeneix BA. Polyethylenimine-based intravenous delivery of transgenes to mouse lung. *Gene Therapy*. 1998; 5 (9): 1291-1295.
185. Forrest ML, Koerber JT, and Pack DW. A degradable polyethylenimine derivative with low toxicity for highly efficient gene delivery. *Bioconjugate Chemistry*. 2003; 14 (5): 934-940.
186. Ahn CH, Chae SY, Bae YH, and Kim SW. Biodegradable poly (ethylenimine) for plasmid DNA delivery. *Journal of Controlled Release*. 2002; 80 (1-3): 273-282.
187. Gosselin MA, Guo WJ, and Lee RJ. Efficient gene transfer using reversibly cross-linked low molecular weight polyethylenimine. *Bioconjugate Chemistry*. 2001; 12 (6): 989-994.
188. Tang GP, Guo HY, Alexis F, Wang X, Zeng S, Lim TM, Ding J, Yang YY, and Wang S. Low molecular weight polyethylenimines linked by beta-cyclodextrin for gene transfer into the nervous system. *Journal of Gene Medicine*. 2006; 8 (6): 736-744.
189. Fu HH, Hu YH, McNelis T, and Hollinger JO. A calcium phosphate-based gene delivery system. *Journal of Biomedical Materials Research Part A*. 2005; 74A (1): 40-48.
190. Gao LZ, Nie L, Wang TH, Qin YJ, Guo ZX, Yang DL, and Yan XY. Carbon nanotube delivery of the GFP gene into mammalian cells. *Chembiochem*. 2006; 7 (2): 239-242.
191. McBain SC, Griesenbach U, Xenariou S, Keramane A, Batich CD, Alton EWFW, and Dobson J. Magnetic nanoparticles as gene delivery agents: enhanced transfection in the presence of oscillating magnet arrays. *Nanotechnology*. 2008; 19 (40): -.
192. Bharali DJ, Klejbor I, Stachowiak EK, Dutta P, Roy I, Kaur N, Bergey EJ, Prasad PN, and Stachowiak MK. Organically modified silica nanoparticles: A nonviral vector for in vivo gene delivery and expression in the brain. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102 (32): 11539-11544.
193. Liu YP, Meyer-Zaika W, Franzka S, Schmid G, Tsoli M, and Kuhn H. Gold-cluster degradation by the transition of B-DNA into A-DNA and the formation of nanowires. *Angewandte Chemie-International Edition*. 2003; 42 (25): 2853-2857.
194. Tan WB, Jiang S, and Zhang Y. Quantum-dot based nanoparticles for targeted silencing of HER2/neu gene via RNA interference. *Biomaterials*. 2007; 28 (8): 1565-1571.

195. Aisawa S, Hirahara H, Ishiyama K, Ogasawara W, Umetsu Y, and Narita E. Sugar-anionic clay composite materials: intercalation of pentoses in layered double hydroxide. *Journal of Solid State Chemistry*. 2003; 174 (2): 342-348.
196. Li WZ, Nesselmann C, Zhou ZH, Ong LL, Ori F, Tang GP, Kaminski A, Lutzow K, Lendlein A, Liebold A, Stamm C, Wang J, Steinhoff G, and Ma N. Gene delivery to the heart by magnetic nanobeads. *Journal of Magnetism and Magnetic Materials*. 2007; 311 (1): 336-341.
197. Li WZ, Ma N, Ong LL, Kaminski A, Skrabal C, Ugurlucan M, Lorenz P, Gatzen HH, Lutzow K, Lendlein A, Putzer BM, Li RK, and Steinhoff G. Enhanced thoracic gene delivery by magnetic nanobead-mediated vector. *Journal of Gene Medicine*. 2008; 10 (8): 897-909.
198. Cai D, Mataraza JM, Qin ZH, Huang ZP, Huang JY, Chiles TC, Carnahan D, Kempa K, and Ren ZF. Highly efficient molecular delivery into mammalian cells using carbon nanotube spearing. *Nature Methods*. 2005; 2 (6): 449-454.
199. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, and Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; 284 (5411): 143-147.
200. Satija NK, Singh VK, Verma YK, Gupta P, Sharma S, Afrin F, Sharma M, Sharma P, Tripathi RP, and Gurudutta GU. Mesenchymal stem cell-based therapy: a new paradigm in regenerative medicine. *Journal of Cellular and Molecular Medicine*. 2009; 13 (11-12): 4385-402.
201. Zvaifler NJ, Marinova-Mutafchieva L, Adams G, Edwards CJ, Moss J, Burger JA, and Maini RN. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Research*. 2000; 2 (6): 477-488.
202. De Bari C, Dell'Accio F, and Luyten FP. Human periosteum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. *Arthritis and Rheumatism*. 2001; 44 (1): 85-95.
203. Nakahara H, Goldberg VM, and Caplan AI. Culture-Expanded Human Periosteal-Derived Cells Exhibit Osteochondral Potential Invivo. *Journal of Orthopaedic Research*. 1991; 9 (4): 465-476.
204. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, and Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood*. 2004; 103 (5): 1669-1675.
205. De Bari C, Dell'Accio F, Tylzanowski P, and Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis and Rheumatism*. 2001; 44 (8): 1928-1942.
206. Brighton CT, Lorich DG, Kupcha R, Reilly TM, Jones AR, and Woodbury RA. The Pericyte as a Possible Osteoblast Progenitor-Cell. *Clinical Orthopaedics and Related Research*. 1992; (275): 287-299.
207. Noth U, Osyczka AM, Tuli R, Hickok NJ, Danielson KG, and Tuan RS. Multilineage mesenchymal differentiation potential of human trabecular bone-derived cells. *Journal of Orthopaedic Research*. 2002; 20 (5): 1060-1069.
208. Osyczka AM, Noth U, Danielson KG, and Tuan RS. Different osteochondral potential of clonal cell lines derived from adult human trabecular bone. *Reparative Medicine: Growing Tissues and Organs*. 2002; 961: 73-77.
209. Boquest AC, Shahdadfar A, Fronsdal K, Sigurjonsson O, Tunheim SH, Collas P, and Brinchmann JE. Isolation and transcription profiling of purified uncultured human stromal stem cells: Alteration of gene expression after in vitro cell culture. *Molecular Biology of the Cell*. 2005; 16 (3): 1131-1141.

210. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, and Hedrick MH. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Engineering*. 2001; 7 (2): 211-228.
211. Polisetty N, Fatima A, Madhira SL, Sangwan VS, and Vemuganti GK. Mesenchymal cells from limbal stroma of human eye. *Molecular Vision*. 2008; 14 (46-53): 431-442.
212. in 'tAnker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FHJ, Willemze R, Fibbe WE, and Kanhai HHH. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood*. 2003; 102 (4): 1548-1549.
213. Martin J, Helm K, Ruegg P, Varella-Garcia M, Burnham E, and Majka S. Adult lung side population cells have mesenchymal stem cell potential. *Cytotherapy*. 2008; 10 (2): 140-151.
214. Young HE, Steele TA, Bray RA, Hudson J, Floyd JA, Hawkins K, Thomas K, Austin T, Edwards C, Cuzzourt J, Duenzl M, Lucas PA, and Black AC. Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. *Anatomical Record*. 2001; 264 (1): 51-62.
215. Bosch P, Musgrave DS, Lee JY, Cummins J, Shuler F, Ghivizzani SC, Evans C, Robbins PD, and Huard J. Osteoprogenitor cells within skeletal muscle. *Journal of Orthopaedic Research*. 2000; 18 (6): 933-944.
216. Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, and Marcacci M. Repair of large bone defects with the use of autologous bone marrow stromal cells. *New England Journal of Medicine*. 2001; 344 (5): 385-386.
217. Diduch DR, Jordan LCM, Mierisch CM, and Balian G. Marrow stromal cells embedded in alginate for repair of osteochondral defects. *Arthroscopy-the Journal of Arthroscopic and Related Surgery*. 2000; 16 (6): 571-577.
218. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, and Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction - (TOPCARE-AMI). *Circulation*. 2002; 106 (24): 3009-3017.
219. Badiavas EV and Falanga V. Treatment of chronic wounds with bone marrow-derived cells. *Arch Dermatol*. 2003; 139 (4): 510-6.
220. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, and Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nature Medicine*. 1999; 5 (3): 309-13.
221. Ringden O, Uzunel M, Rasmusson I, Remberger M, Sundberg B, Lonnies H, Marschall HU, Dlugosz A, Szakos A, Hassan Z, Omazic B, Aschan J, Barkholt L, and Le Blanc K. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation*. 2006; 81 (10): 1390-7.
222. Koc ON, Day J, Nieder M, Gerson SL, Lazarus HM, and Krivit W. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). *Bone Marrow Transplant*. 2002; 30 (4): 215-22.
223. Robey PG and Bianco P. The use of adult stem cells in rebuilding the human face. *Journal of the American Dental Association*. 2006; 137 (7): 961-972.

224. Maitra B, Szekely E, Gjini K, Laughlin MJ, Dennis J, Haynesworth SE, and Koc O. Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. *Bone Marrow Transplantation*. 2004; 33 (6): 597-604.
225. Huang X, Cho S, and Spangrude GJ. Hematopoietic stem cells: generation and self-renewal. *Cell Death and Differentiation*. 2007; 14 (11): 1851-1859.
226. Szilvassy SJ. The biology of hematopoietic stem cells. *Archives of Medical Research*. 2003; 34 (6): 446-60.
227. Li B, Cohen A, Hudson TE, Motlagh D, Amrani DL, and Duffield JS. Mobilized human hematopoietic stem/progenitor cells promote kidney repair after ischemia/reperfusion injury. *Circulation*. 121 (20): 2211-20.
228. Zhou P, Wirthlin L, McGee J, Annett G, and Nolta J. Contribution of human hematopoietic stem cells to liver repair. *Semin Immunopathol*. 2009; 31 (3): 411-9.
229. Krasulova E, Trneny M, Kozak T, and Havrdova E. Autologous Hematopoietic Stem Cells Transplantation and its Current Role in Multiple Sclerosis Treatment. *Ceska a Slovenska Neurologie a Neurochirurgie*. 2009; 72 (3): 227-234.
230. Persons DA, Allay ER, Sawai N, Hargrove PW, Brent TP, Hanawa H, Nienhuis AW, and Sorrentino BP. Successful treatment of murine beta-thalassemia using in vivo selection of genetically modified, drug-resistant hematopoietic stem cells. *Blood*. 2003; 102 (2): 506-513.
231. Martinelli G, Terragna C, Zamagni E, Ronconi S, Tosi P, Lemoli RM, Bandini G, Motta MR, Testoni N, Amabile M, Ottaviani E, Vianelli N, de Vivo A, Gozzetti A, Tura S, and Cave M. Molecular remission after allogeneic or autologous transplantation of hematopoietic stem cells for multiple myeloma. *Journal of Clinical Oncology*. 2000; 18 (11): 2273-2281.
232. Breitbach M, Bostani T, Roell W, Xia Y, Dewald O, Nygren JM, Fries JWU, Tiemann K, Bohlen H, Hescheler J, Welz A, Bloch W, Jacobsen SEW, and Fleischmann BK. Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood*. 2007; 110 (4): 1362-1369.
233. Gugala Z, Olmsted-Davis EA, Gannon FH, Lindsey RW, and Davis AR. Osteoinduction by ex vivo adenovirus-mediated BMP2 delivery is independent of cell type. *Gene Therapy*. 2003; 10 (16): 1289-1296.
234. Zhang XS, Linkhart TA, Chen ST, Peng HR, Wergedal JE, Guttierez GG, Sheng MHC, Lau KHW, and Baylink DJ. Local ex vivo gene therapy with bone marrow stromal cells expressing human BMP4 promotes endosteal bone formation in mice. *Journal of Gene Medicine*. 2004; 6 (1): 4-15.
235. Park J, Ries J, Gelse K, Kloss F, von der Mark K, Wiltfang J, Neukam FW, and Schneider H. Bone regeneration in critical size defects by cell-mediated BMP-2 gene transfer: a comparison of adenoviral vectors and liposomes. *Gene Therapy*. 2003; 10 (13): 1089-1098.
236. Gelse K, von der Mark K, Aigner T, Park J, and Schneider H. Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. *Arthritis and Rheumatism*. 2003; 48 (2): 430-441.
237. Chambers SM, Shaw CA, Gatza C, Fisk CJ, Donehower LA, and Goodell MA. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS Biol*. 2007; 5 (8): e201.
238. Noda S, Ichikawa H, and Miyoshi H. Hematopoietic stem cell aging is associated with functional decline and delayed cell cycle progression.

Biochemical and Biophysical Research Communications. 2009; 383 (2): 210-215.

239. Goncalves MAFV, de Vries AAF, Holkers M, van de Watering MJM, van der Velde I, van Nierop GP, Valerio D, and Knaan-Shanzer S. Human mesenchymal stem cells ectopically expressing full-length dystrophin can complement Duchenne muscular dystrophy myotubes by cell fusion. *Human Molecular Genetics*. 2006; 15 (2): 213-221.
240. Chamberlain JR, Schwarze U, Wang PR, Hirata RK, Hankenson KD, Pace JM, Underwood RA, Song KM, Sussman M, Byers PH, and Russell DW. Gene targeting in stem cells from individuals with osteogenesis imperfecta. *Science*. 2004; 303 (5661): 1198-1201.
241. Shi Q, Hodara V, Butler SD, Thomas CA, Hubbard GB, VandeBerg JL, and Wang XL. Differential bone marrow stem cell mobilization by G-CSF injection or arterial ligation in baboons. *Journal of Cellular and Molecular Medicine*. 2009; 13 (8B): 1896-1906.
242. Klopsch C, Furlani D, Gabel R, Li WZ, Pittermann E, Ugurlucan M, Kundt G, Zingler C, Titze U, Wang WW, Ong LL, Wagner K, Li RK, Ma N, and Steinhoff G. Intracardiac injection of erythropoietin induces stem cell recruitment and improves cardiac functions in a rat myocardial infarction model. *Journal of Cellular and Molecular Medicine*. 2009; 13 (4): 664-679.
243. Lapidot T, Dar A, and Kollet O. How do stem cells find their way home? *Blood*. 2005; 106 (6): 1901-1910.
244. Zhang G, Nakamura Y, Wang X, Hu Q, Suggs LJ, and Zhang J. Controlled release of stromal cell-derived factor-1 alpha in situ increases c-kit+ cell homing to the infarcted heart. *Tissue Engineering*. 2007; 13 (8): 2063-71.
245. Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, and Penn MS. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet*. 2003; 362 (9385): 697-703.
246. Schantz JT, Chim H, and Whiteman M. Cell guidance in tissue engineering: SDF-1 mediates site-directed homing of mesenchymal stem cells within three-dimensional polycaprolactone scaffolds. *Tissue Engineering*. 2007; 13 (11): 2615-24.
247. Dar A, Goichberg P, Shinder V, Kalinkovich A, Kollet O, Netzer N, Margalit R, Zsak M, Nagler A, Hardan I, Resnick I, Rot A, and Lapidot T. Chemokine receptor CXCR4-dependent internalization and resecretion of functional chemokine SDF-1 by bone marrow endothelial and stromal cells. *Nature Immunology*. 2005; 6 (10): 1038-46.
248. Bonig H, Priestley GV, and Papayannopoulou T. Hierarchy of molecular-pathway usage in bone marrow homing and its shift by cytokines. *Blood*. 2006; 107 (1): 79-86.
249. Blades MC, Manzo A, Ingegnoli F, Taylor PR, Panayi GS, Irjala H, Jalkanen S, Haskard DO, Perretti M, and Pitzalis C. Stromal cell-derived factor 1 (CXCL12) induces human cell migration into human lymph nodes transplanted into SCID mice. *Journal of Immunology*. 2002; 168 (9): 4308-4317.
250. Dar A, Kollet O, and Lapidot T. Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. *Experimental Hematology*. 2006; 34 (8): 967-975.

251. McQuibban GA, Butler GS, Gong JH, Bendall L, Power C, Clark-Lewis I, and Overall CM. Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. *Journal of Biological Chemistry*. 2001; 276 (47): 43503-43508.
252. Peterson JT, Li H, Dillon L, and Bryant JW. Evolution of matrix metalloprotease and tissue inhibitor expression during heart failure progression in the infarcted rat. *Cardiovascular Research*. 2000; 46 (2): 307-315.
253. De La Luz Sierra M, Yang F, Narazaki M, Salvucci O, Davis D, Yarchoan R, Zhang HH, Fales H, and Tosato G. Differential processing of stromal-derived factor-1alpha and stromal-derived factor-1beta explains functional diversity. *Blood*. 2004; 103 (7): 2452-9.

List of publications

1. **Wang W**, Li W, Ou L, Flick E, Mark P, Nesselmann C, Lux CA, Gatzen HH, Kaminski A, Liebold A, Lützow K, Lendlein A, Li RK, Steinhoff G and Ma N. Polyethylenimine-mediated gene delivery into human bone marrow mesenchymal stem cells from patients. *Journal of Cellular and Molecular Medicine.* 2010 Jul 13. [Epub ahead of print] (2009 I.F. = 5.228)

2. Ou L, Li W, Zhang Y, **Wang W**, Liu J, Sorg H, Furlani D, Gäbel R, Mark P, Klopsch C, Wang L, Lützow K, Lendlein A, Wagner K, Klee D, Liebold A, Li RK, Kong D, Steinhoff G and Ma N. Intracardiac injection of matrigel induces stem cell recruitment and improves cardiac functions in a rat myocardial infarction model. *Journal of Cellular and Molecular Medicine.* 2010 May 14. [Epub ahead of print] (2009 I.F. = 5.228)

3. Delyagina E, Ma N, **Wang W**, Zhang Y, Kuhlo A, Flick E, Gatzen H, Steinhoff G and Li W. Magnetically guided transfection in suspension cells with PEI 25kDa conjugated to magentic nanoparticles. *Biomedizinische Technik/Biomedical Engineering*. 2010; 55 (Suppl.1): 37-39.

4. Delyagina E, Ma N, **Wang W**, Zhang Y, Kuhlo A, Flick E, Gatzen H, Zhang F, Burkel E, Steinhoff G and Li W. Carbon nanotube – mediated polyethylenimine for gene delivery. *Biomedizinische Technik/Biomedical Engineering*. 2010; 55 (Suppl.1): 40-41.

5. **Wang W**, Li W, Ong LL, Lutzow K, Lendlein A, Furlani D, Gabel R, Kong D, Wang J, Li RK, Steinhoff G and Ma N. Localized and sustained SDF-1 gene release mediated by fibronectin films: A potential method for recruiting stem cells. *The International Journal of Artificial Organs*. 2009 Mar; 32(3):141-9. (2008 I.F. = 1.299)

6. **Wang W**, Li W, Ma N and Steinhoff G. Novel biodegradable copolymer suitable for delivering nucleic acid materials into cells. [European patent filed]

7. Emmrich S, **Wang W**, John K, Li W and Pützer BM. Antisense gapmers selectively suppress individual oncogenic p73 splice isoforms and inhibit tumor growth in vivo. *Molecular Cancer*. 2009 Aug 11; 8:61. (2008 I.F. = 5.362)

8. Klopsch C, Furlani D, Gäbel R, Li W, Pittermann E, Ugurlucan M, Kundt G, Zingler C, Titze U, **Wang W**, Ong LL, Wagner K, Li RK, Ma N and Steinhoff G. Intracardiac injection of erythropoietin induces stem cell recruitment and improves cardiac functions in a rat myocardial infarction model. *Journal of Cellular and Molecular Medicine*. 2009 Apr; 13(4):664-79. (2007 I.F. = 6.807)

9. Furlani D, Ugurlucan M, Ong L, Bieback K, Pittermann E, Westien I, **Wang W**, Yerebakan C, Li W, Gäbel R, Li RK, Vollmar B, Steinhoff G and Ma N. Is the intravascular administration of mesenchymal stem cells safe? Mesenchymal stem cells and intravital microscopy. *Microvascular Research*. 2009 May; 77(3):370-6. Epub 2009 Feb 26. (2008 I.F. = 3.000)

10. **Wang W,** Li W, Ong LL, Furlani D, Kaminski A, Liebold A, Lützow K, Lendlein A, Wang J, Li RK, Steinhoff G and Ma N. Localized SDF-1alpha gene release mediated by collagen substrate induces CD117+ stem cell homing. *Journal of Cellular and Molecular Medicine*. 2010 Jan; 14(1-2):392-402. Epub 2008 Dec 24. (2007 I.F. = 6.807)

11. Furlani D, Klopsch C, Gäbel R, Ugurlucan M, Pittermann E, Klee D, Wagner K, Li W, **Wang W**, Ong LL, Nizze H, Titze U, Lützow K, Lendlein A, Steinhoff G and Ma N. Intracardiac erythropoietin injection reveals antiinflammatory potential and improved cardiac functions detected by forced swim test. *Transplantation Proceedings*. 2008 May; 40(4):962-6. (2007 I.F. = 1.027)

12. **Wang W**, Chang Z, Wang M and Zhang Z. Effect of carboxyl on vulcanization and mechanical properties of carboxylated acrylic rubber prepared by Co-60-gamma-ray-induced polymerization. *Journal of Applied Polymer Science*. 2006; 102:5587-5594. (2008 I.F. = 1.187)

13. Geng H, Wang M, Ge X and **Wang W**. Preparation and properties of the self-crosslinked acrylic rubber via gamma ray initiated emulsion polymerization. *Polymer Engineering & Science*. 2006; 46:1748-1753. (2008 I.F. = 1.245)

List of abstracts and presentations

1. Delyagina E, Ma N, **Wang W**, Zhang Y, Kuhlo AL, Flick E, Gatzen HH , Steinhoff G and Li W. PEI 600 Da conjugated to magnetic nanobeads as a non-viral vector for gene delivery. BMT 2010 / 44. DGBMT Jahrestagung 3-Länder-Tagung D-A-CH, October 5-8, 2010. Rostock, Germany.

2. **Wang W**, Li W, Ou L, Mark P, Nesselmann C, Lux C, Ma N and Steinhoff G. Polyethylenimine-mediated gene delivery into bone marrow derived mesenchymal stem cells from patients. 3rd International Congress on Stem Cells and Tissue Formation, July 11-14, 2010. Dresden, Germany.

3. Steinhoff G, Li W, Delyagina E, **Wang W** and Ma N. Enhanced thoracic gene delivery by magnetic nanobead mediated-vector. The 8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers, May 25-29, 2010. Rostock, Germany. (Invited Talk)

4. Delyagina E, Ma N, **Wang W**, Kuhlo AL, Zhang Y, Flick E, Gatzen HH, Steinhoff G and Li W. PEI 600Da conjugated to magnetic beads as a non-viral vector for gene delivery. The 8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers, May 25-29, 2010. Rostock, Germany.

5. Ou L, Zhang Y, Li W, Furlani D, Gäbel R, **Wang W**, Wang L, Liebold A, Steinhoff G and Ma N. Directional migration of c-Kit+ cells to infarcted myocardium by anti-c-kit antibody. 39th Annual meeting of German society for thoracic and cardiovascular surgery, February 14-17, 2010. Stuttgart, Germany.

6. Pittermann E, Furlani D, Klopsch C, Gäbel R, Li W, **Wang W**, Wagner K, Steinhoff G and Ma N. Erythropoietin-α patterns of stem cell chemoattractant genes regulation in cardiac myocytes and endothelial cells. 8th International Luebeck Conference on the Pathophysiology and Pharmacology of Erythropoietin and other Haematopoietic Growth Factors, July 30 – August 1, 2009. Lübeck, Germany.

7. **Wang W**, Li W, Flick E, Li X, Lützow K, Lendlein A, Gatzen HH, Steinhoff G and Ma N. Poly(ethyleneimine) based biodegradable cationic polymer as non-viral gene delivery vector. International Conference on Materials for Advanced Technologies 2009 & International Union of Materials Research Societies-International Conference in Asia 2009, June 28-July 3, 2009. Singapore.

8. **Wang WW**, Li WZ, Ong L, Lutzow K, Lendlein A, Furlani D, Gäbel R, Kong DL, Wang J, Steinhoff G and Ma N. Fibronectin Films-Mediated SDF-1 Gene Release Recruits Stem Cells. *Molecular Therapy*. 2009; 17: S269-S269.

9. Ou LL, Li WZ, Zhang Y, Gäbel R, Furlani D, **Wang WW**, Wang L, Klopsch C, Mark P, Kong DL, Li RK, Steinhoff G and Ma N. Local Injection of Anti-c-kit Antibody Enhanced Myocardial Homing of Stem Cells and Improved Heart Function. *Circulation*. 2009; 120 (18): S739-S739.

10. Ou LL, Li WZ, Zhang Y, Gäbel R, Furlani D, **Wang WW**, Wang L, Klopsch C, Peter M, Wagner K, Kong DL, Steinhoff G and Ma N. Injection of matrigel induces stem cell recruitment and improves cardiac functions. *Human Gene Therapy*. 2009; 20 (11): 1457-1458.

11. Gäbel R, Klopsch C, Furlani D, Li WZ, Yerebakan C, **Wang WW**, Pittermann E, Dryndao A, Lenz S, Li RK, Ma N and Steinhoff G. Intramyocardial Administration of Erythropoietin Promotes Cell Proliferation, Induces Early Angiogenesis and Attenuates Cardiac Remodeling. *Circulation*. 2008; 118 (18): S875-S875. Annual meeting of the American Heart Association, Scientific Sessions, November 8-12, 2008. New Orleans, USA.

12. **Wang W**, Li W, Ong L, Furlani D, Gäbel R, Ugurlucan M, Klopsch C, Lorenz P, Lutzow K, Lendlein A, Ma N and Steinhoff G. Collagen and Fibronectin Substrates Mediated Local SDF-1 Gene Expression Induces Stem Cells Homing. *International Journal of Artificial Organs*. 2008; 31 (7): 606-606.

13. **Wang W**, Li W, Ong LL, Furlani D, Gäbel R, Ugurlucan M, Klopsch C, Lorenz P, Lützow K, Lendlein A, Steinhoff G and Ma N. Collagen and fibronectin substrates mediated local SDF-1 gene expression induces stem cells homing. European Society for Artificial Organs (ESAO): Annual Meeting, September 3-6, 2008. Geneva, Switzerland.

14. Furlani D, Ugurlucan M, Ong LL, **Wang W**, Pittermann E, Kaminski A, Donndorf P, Yerebakan C, Ma N, Vollmar B and Steinhoff G. Mesenchymal Stem Cells Following Intraarterial Injection: an Intravital Microscopy Study in a Rodent Model. 2nd International Congress on Stem Cells and Tissue Formation, July 6-9, 2008. Dresden, Germany.

15. Klopsch C, Furlani D, Gäbel R, Wagner K, **Wang W**, Ong LL, Li W, Nizze H, Titze U, Lendlein A, Lützow K, Li RK, Ma N and Steinhoff G. Intrakardiale Injektion von Epoetin induziert Hochregulierung von Genen chemoattraktiver Moleküle zur Rekrutierung von Stammzellen nach akutem Myokardinfarkt in Ratten. 37. Rostocker Gespräche über kardiovaskuläre Funktion und Hypertonie, May 31, 2008. Rostock, Germany.

16. Ugurlucan M, Furlani D, Pittermann E, Ong LL, Yerebakan C, Gäbel R, Klopsch C, **Wang W**, Li W, Ma N and Steinhoff G. Is Intravascular Transplantation of Mesenchymal Stem Cells Safe? *Molecular Therapy*, Volume 16, Supplement 1. Annual meeting of the American Society of Gene Therapy, May 28-June 1, 2008. Boston, Massachusetts, USA.

17. Furlani D, Klopsch C, Gäbel R, Wagner K, Ugurlucan M, Li W, **Wang W**, Ong LL, Liebold A, Ma N and Steinhoff G. Intracardiac injection of epoetin upregulates SDF-1 gene expression promoting myocardial regeneration in a rat myocardium infarction. 5 Gemeinsamen Jahrestagung der Deutschen, Österreichischen und Schweizer Gesellschaften für Thorax-, Herz- und Gefässchirurgie (D-A-CH), February 17 – 20, 2008. Innsbruck, Austria.

18. **Wang W**, Li W, Ong LL, Lorenz P, Lützow K, Lendlein A, Ma N and Steinhoff G. Localized SDF-1 gene release mediated by collagen and fibronectin substrates

induces stem cells homing. 2007 Annual Conference of Tissue Engineering and Regenerative Medicine International Society – Asian Pacific Region (2007 TERMIS - AP), December 3-5, 2007. Tokyo, Japan.

19. Klopsch C, Furlani D, Gäbel R, Wagner K, **Wang W**, Ong LL, Li W, Nizze H, Titze U, Lendlein A, Lutzow K, Li RK, Ma N and Steinhoff G. Intracardiac injection of Epoetin-alpha upregulates stem cell chemoattractant gene expression in a rat myocardial infarction model. *Circulation*. 2007; 116 (16): 212-213. Annual meeting of the American Heart Association, Scientific Sessions, November 4-7, 2007. Orlando, USA.

20. Klopsch C, Furlani D, Gäbel R, Wagner K, **Wang W**, Ong LL, Li W, Nizze H, Titze U, Peuster M, Steinhoff G and Ma N. Intramyokardiale Injektion von Epoetin-alpha beeinflusst den Heilungsprozess nach akutem Herzinfarkt in Ratten, verbessert Herzfunktion und verringert pulmonale Hypertension. 16. Jahrestagung der Deutschen TransplantationsGesellschaft, October11-13, 2007. Mainz, Germany.

21. Furlani D, Klopsch C, Gäbel R, Wagner K, Ugurlucan M, **Wang W**, Ong LL, Li W, Nizze H, Titze U, Liebold A, Ma N and Steinhoff G. Intracardiac Injection of Epoetin-a Upregulates SDF-1 Gene Expression Promoting Myocardial Regeneration in a Rat Myocardial Infarction. Konferenz Regenerative Medizin Mikro und Nanotechnologien im Bereich der Regenerativen Medizin, October 10, 2007. Hannover, Germany.

22. Furlani D, Klopsch C, Gäbel R, Wagner K, Li W, Ugurlucan M, **Wang W**, Ong LL, Nizze H, Titze U, Ma N and Steinhoff G. Intracardiac Injection of Epoetin-a Upregulates Stem Cell Chemoattractant Gene Expression Promoting Myocardial Regeneration in a Rodent Ischemia Model. Annual meeting of German Society for Stem Cell Research, October, 2007. Würzburg, Germany.

23. Klopsch C, Furlani D, Gäbel R, Wagner K, **Wang W**, Ong LL, Li W, Nizze H, Titze U, Lendlein A, Lützow K, Li RK, Ma N and Steinhoff G. Intracardiac injection of Epoetin-alpha upregulates stem cell chemoattractant gene expression in a rat myocardial infarction model. Rostocker Gespräche über kardiovaskuläre Funktion und Hypertonie, June 9, 2007. Rostock, Germany.

24. Li W, Ma N, Luetzow K, Ong LL, **Wang W**, Liebold A, Lendlein A and Steinhoff G. Controlled and sustained gene release from polymer DNA matrices scaffold for heart valve tissue engineering. Annual Meeting of the American Society of Gene Therapy, May 30-June 3, 2007. Seattle, USA.

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Selbständigkeitserklärung

Ich versichere hiermit, dass ich die vorliegende Arbeit mit dem Thema:
"Polymer mediated gene delivery for adult stem cell therapy"
("Polymervermittelter Gentransfer für die Therapie mit adulten Stammzellen")
selbstständig verfasst und keine anderen Hilfsmittel als die angegebenen benutzt habe. Die Stellen, die anderen Werken dem Wortlaut oder dem Sinn nach entnommen sind, habe ich in jedem einzelnen Fall durch Angabe der Quelle kenntlich gemacht.
Ich erkläre hiermit weiterhin, dass ich meine wissenschaftlichen Arbeiten nach den Prinzipien der guten wissenschaftlichen Praxis gemäß der gültigen "Satzungen der Universität Rostock zur Sicherung guter wissenschaftlicher Praxis" angefertigt habe.

Rostock, den _____

(Unterschrift)

Reprints of publications included in this dissertation

1. **Wang W**, Li W, Ou L, Flick E, Mark P, Nesselmann C, Lux CA, Gatzen HH, Kaminski A, Liebold A, Lützow K, Lendlein A, Li RK, Steinhoff G and Ma N. Polyethylenimine-mediated gene delivery into human bone marrow mesenchymal stem cells from patients. *Journal of Cellular and Molecular Medicine.* 2010 Jul 13. [Epub ahead of print] (2009 Impact factor = 5.228)

2. Ou L, Li W, Zhang Y, **Wang W**, Liu J, Sorg H, Furlani D, Gäbel R, Mark P, Klopsch C, Wang L, Lützow K, Lendlein A, Wagner K, Klee D, Liebold A, Li RK, Kong D, Steinhoff G and Ma N. Intracardiac injection of matrigel induces stem cell recruitment and improves cardiac functions in a rat myocardial infarction model. *Journal of Cellular and Molecular Medicine.* 2010 May 14. [Epub ahead of print] (2009 Impact factor = 5.228)

3. Emmrich S, **Wang W**, John K, Li W and Pützer BM. Antisense gapmers selectively suppress individual oncogenic p73 splice isoforms and inhibit tumor growth in vivo. *Molecular Cancer*. 2009 Aug 11; 8:61. (2008 Impact factor = 5.362)

4. **Wang W**, Li W, Ong LL, Lutzow K, Lendlein A, Furlani D, Gabel R, Kong D, Wang J, Li RK, Steinhoff G and Ma N. Localized and sustained SDF-1 gene release mediated by fibronectin films: A potential method for recruiting stem cells. *The International Journal of Artificial Organs*. 2009 Mar; 32(3):141-9. (2008 Impact factor = 1.299)

5. **Wang W,** Li W, Ong LL, Furlani D, Kaminski A, Liebold A, Lützow K, Lendlein A, Wang J, Li RK, Steinhoff G and Ma N. Localized SDF-1alpha gene release mediated by collagen substrate induces CD117+ stem cell homing. *Journal of Cellular and Molecular Medicine*. 2010 Jan; 14(1-2):392-402. Epub 2008 Dec 24. (2007 Impact factor = 6.807)

Publication 1

Polyethylenimine-mediated gene delivery into human bone marrow mesenchymal stem cells from patients

Wang W, Li W, Ou L, Flick E, Mark P, Nesselmann C, Lux CA, Gatzen HH, Kaminski A, Liebold A, Lützow K, Lendlein A, Li RK, Steinhoff G and Ma N

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Publication 2

Intracardiac injection of matrigel induces stem cell recruitment and improves cardiac functions in a rat myocardial infarction model

Ou L*, Li W*, Zhang Y*, **Wang W***, Liu J, Sorg H, Furlani D, Gäbel R, Mark P, Klopsch C, Wang L, Lützow K, Lendlein A, Wagner K, Klee D, Liebold A, Li RK, Kong D, Steinhoff G and Ma N

Journal of Cellular and Molecular Medicine
2010 May 14. [Epub ahead of print]

* Equal contribution

Available at: http://www.ncbi.nlm.nih.gov/pubmed/20477905

Publication 3

Antisense gapmers selectively suppress individual oncogenic p73 splice isoforms and inhibit tumor growth *in vivo*

Emmrich S, **Wang W**, John K, Li W and Pützer BM

Molecular Cancer
2009 Aug 11; 8:61.

Available at: http://www.ncbi.nlm.nih.gov/pubmed/19671150

Publication 4

Localized and sustained SDF-1 gene release mediated by fibronectin films: A potential method for recruiting stem cells

Wang W, Li W, Ong LL, Lutzow K, Lendlein A, Furlani D, Gabel R, Kong D, Wang J, Li RK, Steinhoff G and Ma N

The International Journal of Artificial Organs
2009 Mar; 32(3):141-9.

Available at: http://www.ncbi.nlm.nih.gov/pubmed/19440989

Publication 5

Localized SDF-1alpha gene release mediated by collagen substrate induces CD117^{+} stem cell homing

Wang W, Li W, Ong LL, Furlani D, Kaminski A, Liebold A, Lützow K, Lendlein A, Wang J, Li RK, Steinhoff G and Ma N

Journal of Cellular and Molecular Medicine
2010 Jan; 14(1-2):392-402. Epub 2008 Dec 24.

Available at: http://www.ncbi.nlm.nih.gov/pubmed/19413887