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REVIEW PAPER

Gene Delivery Platforms

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Abstract One of the key challenges in the experimental and therapeutic use of gene delivery agents is the development of methods that can efficiently deliver nucleic acids into living systems. During the past decade, the development of effective and safe gene delivery systems has been intensively investigated. This review summarizes the current state of gene delivery methods based on viral and non-viral agents.

Keywords: gene delivery system, GDS, nanoparticle, viral GDS, non-viral GDS

1. Introduction

A gene delivery system (GDS) is a special purpose conjugate that consists of carry-over material and nucleic acids (payload). The carry-over material must have the ability to deliver nucleic acids through the cell membrane, which serves as a protective barrier to harmful foreign materials that may interfere with cellular function. Thus, carry-over material of a GDS can pass through the cell membrane, and in some instances, enter the nucleus. Over the past few decades, the development of gene delivery systems has emerged as a key area of research, with the hope of producing promising gene therapies in the future (for a recent review, see ref [1,2]).

GDSs can be classified into two categories based on the origin of the gene carrier. First, viral gene delivery systems use recombinant viruses as gene carriers. Viral GDSs exhibit efficient delivery of genes into the target cells, however, there are limitations, including a strong immune response triggered by the expression of viral genes, oncogenic insertions into the genome, and unstable maintenance of viruses in the host cell. Several viral GDSs have been reported such as viral vectors based on retrovirus, lentivirus, adeno-virus, adeno-associated vectors, and herpes simplex virus. A second type of GDS is a non-viral gene delivery system using physical (carrier-free gene delivery) and chemical approaches (synthetic vector-based gene delivery). Despite recent developments in GDS, their application has been hindered by problems such as low transduction efficiency of target cells, cytotoxicity, and mutagenicity of the chromosomal DNA (for a recent review, see ref [1,2]).

In order to deliver genes into cells, several barriers need to be overcome including the cell membrane barrier, endolysosomal entrapment, cytosolic sequestration, and nuclear exclusion. Cellular attachment of the GDS can be mediated by non-specific hydrophobic or electrostatic interactions of specific receptor proteins in the cellular membrane. These interactions eventually result in trans-membrane signaling, activation of the endocytotic machinery and, subsequently, endocytosis of GDS. Direct fusion between the cell membrane and/or fluid phase endocytosis may also account for the cellular uptake of the complex. Cellular uptake of the GDS begins in the early endosomal vesicles. These early endosomes are fused with sorting endosomes [3], and their contents are subsequently transferred to late endosomes. Late endosomal vesicles are acidified (pH 5 ~ 6) by the inversion of a membrane-bound proton-pump ATPase. Acidified late endosomal vesicles are fused

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Table 1. Advantages and disadvantages of gene delivery systems

	Vector	Advantages	Disadvantages
Viral vector	Retrovirus	▪ Stable integration into host genome ▪ Stable and long-term transgene expression ▪ Relatively easy manipulation of viral genome for vector engineering	▪ Medium insertion capacity for transgene (< 7 ~ 8 kb) ▪ Difficult targeting of viral infection ▪ Random integration into host genome ▪ Instability of vectors ▪ Infect into dividing cells only
	Lentivirus	▪ Stable transgene expression level ▪ Broad host range affinity of infectivity ▪ Infect into dividing and non-dividing cells	▪ Medium insertion capacity for transgene (< 7 ~ 8 kb) ▪ Potential insertional mutagenesis ▪ Safety concerns about HIV
	Adenovirus	▪ Broad host range affinity of infectivity ▪ Infect into dividing and non-dividing cells	▪ Medium insertion capacity for transgene (< 7 ~ 8 kb) ▪ Transient gene expression ▪ Strong immune response to viral protein
	Adeno-associated virus (AAV)	▪ Broad host range affinity of infectivity ▪ Infect into dividing and non-dividing cells ▪ Low immune response and nonpathogenic	▪ Low insertion capacity for transgene (< 4 kb) ▪ Slow onset of gene expression ▪ Inefficient large-scale virus production
	Herpesvirus	▪ High insertion capacity for transgene (>30 kb) ▪ Broad host range affinity of infectivity ▪ Long-term transgene expression ▪ Natural tropism to neuronal cells	▪ Potential toxicity and risk of recombination ▪ No viral integration into host genome
Non-viral vector	Naked DNA	▪ Simple manipulation and lack of toxicity	▪ Low levels of gene expression ▪ Instability of DNAs ▪ Retargeting transfection very difficult
	Cationic polymer-based GDS	▪ Highly effective *in vitro* ▪ Low to medium high for local and systemic gene delivery	▪ Molecular weight dependent toxicity and transfection activity ▪ Acute immune responses
	Lipid-based GDS	▪ Low to medium high efficiency *in vitro* and *in vivo* ▪ Low toxicity	▪ Low activity *in vivo*
	Nanoparticle	▪ High transfection efficiency ▪ Easy to incorporate different functions on a single particle ▪ Size is tunable from 1nm to 200nm	▪ No clinical experience
	Electroporation	▪ Higher level of gene expression than DNA alone ▪ Effectively delivered large amount of gene (>100 kb) ▪ Long-term transgene expression ▪ Less variation in efficiency across species	▪ Limited effective working range of electrode ▪ Required surgical procedure when use for non-topical application ▪ Can result in irreversible tissue damage
	Gene gun	▪ Ideal method for gene transfer to skin, mucosa, or surgically exposed tissues ▪ Simple and effective method	▪ Low insertion capacity for transgene ▪ Limited application into multiple tissue

with lysosomes and the endosomal content is then relocated to the lysosomes, which are further acidified (pH ~ 4.5). The lysosomes contain various nucleases that promote the degradation of nucleic acids. The genes or gene-carrier conjugates must be released from the endosome into the cytosol in order to protect the genes from lysosomal degradation. Thus, release of the GDS from the endosome is crucial for efficient gene transfer.

Boussif *et al*. hypothesized that polyethlylenimines (PEIs), which were used as gene delivery carriers, are released from the endosome by the "proton sponge effect" [4]. PEIs have a high buffering capacity, which induces increased influx of protons, followed by water and chloride ions. The resulting osmotic swelling can lead to the creation of a positive charge and/or enhanced membrane interaction with PEIs, which ruptures the endosomal membrane. Another mechanism for endosomal escape is "membrane destabilization". There is sufficient evidence that liposomal complexes (lipoplexes) are able to destabilize the endosomal/lysosomal membranes [5] and it was demonstrated that

efficient cationic lipid formulations can perforate the endosomal membrane, while less efficient lipid formulations cannot [6].

Most non-viral gene vectors cannot readily cross the cellular membrane due to large size and hydrophilicity. Endocytosis is the main mechanism for cellular uptake of non-viral vectors. Endocytosis can be classified into two broad categories, phagocytosis (cell eating, uptake of large particles) and pinocytosis (cell drinking, uptake of small particles, fluid, and solutes). There are 5 categories of endocytic pathways used in gene delivery; clathrin-medicated endocytosis (CME), caveolae-mediated endocytosis, macropinocytosis, phagocytosis, and receptor-mediated endocytosis.

When DNA is delivered into eukaryotic cells, it normally has to enter the nucleus to function. However, this process is not easy, unless the nuclear compartment is broken down during mitosis. Gene delivery to cells by carriers is followed by dissociation of DNA from the carriers. The DNA does not stay 'naked' or uncomplexed but binds quickly to a number of intracellular proteins, cationic peptides and polyamines. A protein that is targeted to the nucleus contains a relatively short sequence known as the 'nuclear localization signal (NLS)'. Cargo proteins bearing a NLS consisting of clusters of basic residues are bound and imported by a class of proteins known as karyopherins (importins α and β) that are soluble in the nuclear pore complex (NPC). Another sequence, termed the 'SV40 DNA nuclear targeting sequence (DTS)', efficiently mediates the nuclear import of plasmid DNA in mammalian cells [7].

2. Viral Vectors for Gene Delivery Systems

Viral vectors have been developed as potential carriers of genes due to their natural ability to transport genes into the nucleus of the host cells while evading degradation by lysosomes. Constructing a viral vector for use as a GDS is accomplished by producing a recombinant virus that lacks replication capability, but maintains the ability to infect cells. Starting from retroviruses, several viral vectors such as adenoviruses, lentiviruses, adeno-associated viruses (AAV), and herpes simplex viruses were used to develop GDS. Viruses often utilize the mechanism for entry including fusion of the viral membrane at the cell surface, or receptor-mediated endocytosis. Non-enveloped viral vectors are incorporated into the cell *via* translocation across the lipid bilayer. Enveloped viruses interact with cell receptors *via* the action of membrane-associated viral glycoproteins that project beyond the viral envelope. Viral vectors are still the most efficient GDSs because of their ability to infect a high proportion of cells while carrying transgenes in their modified genomes. However, viral vectors have potential safety concerns related to virus-mediated target cell killing.

2.1. Retroviral vectors

Retroviruses, the first types of viral vectors to be explored [8], are single-stranded RNA viruses that use their own reverse transcriptases. The genomic RNA is capped and polyadenylated, and is composed of four genes, *gag, pro, pol* and *env*, which encode for structural capsid proteins, viral protease, integrase and viral reverse transcriptase, and envelop glycoproteins, respectively. In addition to these genes, retroviruses have long terminal repeats (LTRs) at each end, which act as promoters. One widely used retroviral vector is derived from the Moloney murine leukemia virus (Mo-MLV).

To use retroviruses for GDS, all retroviral genes except LTR sequences should be removed and replaced with markers or desired genes, or both. This deletion of *gag*, *env* or *pol* genes makes the vector replication defective, unless complemented in the packaging cell line. LTRs are necessary for a GDS because infected cells are not able to express any viral proteins without these sequences. However, LTRs can activate the provirus in the infected cell and a replication-competent virus can be generated during propagation of the vectors. To avoid the inadvertent spread of the therapeutic retroviral vector to nontarget tissues by generation of a replication-competent virus, retrovirus-derived vectors containing deletions in the 3'-LTR called self-inactivating vectors have been developed to ensure the transcriptional inactivation of the provirus in the infected cell [9].

The advantages of retroviral vectors include their ability to integrate into the host genome and therefore sustain heterologous gene expression at high levels and for long periods. However, the instability of these vectors makes insertional mutagenesis possible by random viral integration into the host genome [1]. In addition, retroviral vectors cannot infect non-dividing cells, which restricts their potential applications. Therefore, the retroviral vectors are suitable for *ex vivo* gene therapy. For instance, these vectors were successfully used for transducing CD34+ bone marrow hematopoietic stem cells and peripheral blood lymphocytes [10]. Despite all the disadvantages, retroviral gene delivery systems have been used in many clinical trials due to the absence of alternative methods that offer efficient and safe gene delivery [11].

2.2. Lentiviral vectors

Although lentiviruses are types of retroviruses, they have special features that distinguish them from general retroviruses. Many of the lentiviral vectors used in gene therapy are based on the human immunodeficiency virus

(HIV), which is known to cause human acquired immune deficiency syndrome (AIDS). One major advantage of lentiviruses is their ability to infect and integrate into non-dividing cells, a feature that is lacking in other retroviral vectors [12]. Use of a lentiviral vector in the same manner as a retroviral vector system consequently achieved long-term expression of the transferred gene. Recently developed lentiviral vectors have resulted in efficient transduction of hepatocytes [13]. In this study, there is no evidence that the HIV-based vector is stably integrated into the host genome although integration of the vector genome in the liver DNA of immunodeficient mice and stable expression of the marker green fluorescent protein (GFP)-encoding gene were observed [13]. Therefore, extensive studies are needed to address the biosafety of lentiviral vectors due to their origin before application in human gene therapy.

2.3. Adenoviral vectors

Adenoviruses are icosahedral, non-enveloped double-stranded DNA viruses, which are associated with cold or mild flu-like respiratory diseases. They are the best described and the most used systems for gene transfer, originally prepared to overcome the limitations of retroviral vectors [1]. Three generations of adenoviral vectors have been investigated in order to decrease cytopathic effects and immune responses. The first generation has a deletion in the E1 region that ensures viral replication and DNA synthesis. The first generation of adenoviral vectors still presented notable immunogenicity and low-level replication. These led to the manipulation of other viral regions either E2 or E4 [14]. Second generation has significantly reduced cytopathic effects and immune responses. Third generation called "gutless vectors" was developed by deleting all viral genes except inverted terminal repeats and the packaging signal, which provided highly reduced immunogenicity and capacity up to 34 kb [15]. Recombinant adenoviral vectors have the following advantages: ability to infect a wide variety of cell types, high gene transfer efficiency, and ability to infect both dividing and non-dividing cells, lack of integration into the host genome, easy manipulation, and high titers. The disadvantages of these vectors *in vivo* are the limited duration of the transgene expression, immune response elicited against the viral vector, and expression of vector-encoded proteins in infected cells. For these reasons, recent studies are focused on inhibiting inflammatory responses against adenoviral vectors and circumventing the degradation of adenoviral particles. A promising approach in this area is to use vectors with the capsid proteins derived from different adenoviral serotypes [16]. In cancer therapy, immunogenicity can be beneficial as it stimulate the patient's immune system against tumor cells.

2.4. Adeno-associated viral vectors

Adeno-associated viruses (AAV) are dependoviruses belonging to the parvoviridae family. AAV can replicate with a helper virus like adenovirus or occasionally herpes simplex virus (HSV). They have a simple structure; a short, linear, single-stranded DNA genome composed of two open reading frames, *rep* (regulation) and *cap* (capsid), and two small inverted terminal repeats (ITRs). Unlike retroviruses, which are of murine origin, the adeno-associated viral transfer systems are derived from human viruses and therefore may be more feasible for human gene therapy. It has been reported that a single intracellular injection of a rAAV vector carrying the mouse *leptin* gene produces long-term corrections of endocrine and metabolic defects [17]. As the infection of human cells results in long-term persistence of a proviral genome, AAV vectors can be used for the long-lasting expression of a therapeutic gene [1]. The main advantages of AAV vector systems are: high stability, heat-inactivation resistance, wide host range, and broad tissue tropism. Recent studies indicate that the main limitation of small packaging capacity will also be overcome [18]. AAV vectors have been used for *ex vivo* transduction of B cells from chronic lymphocytic leukemia, cystic fibrosis, and hemophilia B [19].

2.5. Herpes simplex viral vectors

Herpes simplex virus-1 (HSV-1) is an enveloped, double-stranded DNA virus with a large genome (~152 kb) encoding more than 80 genes. HSV-1 is able to infect cells lytically or it can establish latency. Two types of HSV-1 vectors have been developed, the recombinant HSV-1 vectors and the amplicon vectors. The main advantage of HSV-based vectors is their large cloning capacity for foreign genes (30 ~ 40 kb) since the genome of HSV is one of the biggest among viral vectors and some genes can be deleted and this enables the introduction of a very large gene or even of several smaller genes [20]. On the other hand, after entering a latent state, the HSV turns off the expression of all genes, including the gene of interest. Only one small region in the HSV genome, the LAT region, stays active during latency. This limitation can be overcome by inserting the foreign gene into the LAT region [21]. HSV vectors are particularly attractive for treatment of neurological disorders where long-term expressions of therapeutic genes are required. Replication-defective HSV vectors have also been used for targeting muscular delivery [2].

3. Non-viral Gene Delivery Systems

As viral vector-based GDSs have immunological and

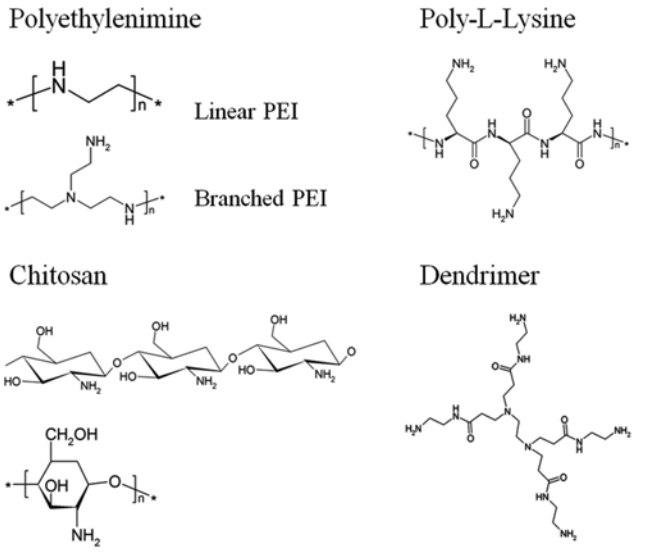

Fig. 1. Representative chemical structures of cationic polymer gene delivery systems**.** Polyethylenimine, Polylysine, and Dendrimer are synthetic polycations. Chitosan is obtained by alkaline hydrolysis of chitin, a naturally occurring seaweed polysaccharides.

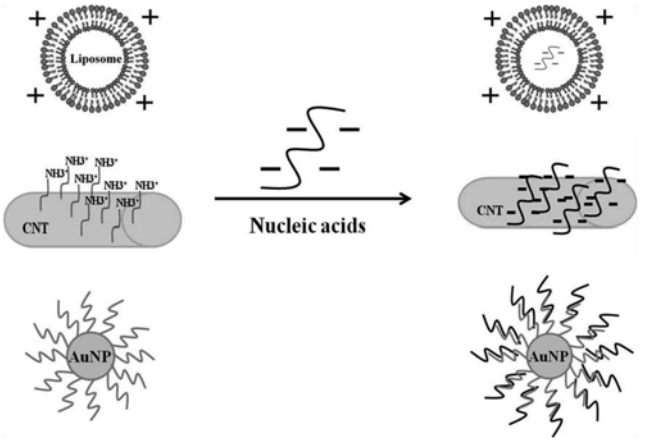

Fig. 2. Scheme of nanoparticles for gene delivery system. Gene delivery systems based on liposome, carbon nanotube (CNT), and gold nanoparticle (AuNP) are shown.

cytotoxic disadvantages, non-viral vectors have attracted increasing attention as alternative carriers. Non-viral GDS must carry genetic material payloads, pass cellular barriers without causing immune responses, release genetic material into the nucleus, and, if possible, allow visualization of GDS in the cells. The transfection efficiency of non-viral synthetic GDSs has improved during the last decade, but their efficiency is still low compared to those of viral vectors. Methods of non-viral GDS have been developed using physical and chemical approaches. Physical methods, including needle injection, gene gun, electroporation, use a physical force that permeates the cell membrane and facilitates gene transfer. Chemical methods use synthetic or naturally occurring compounds as carriers to deliver the transgene into cells. Non-viral synthetic vectors include cationic polymer complexes (polyplexes), lipid complexes (lipoplexes), and inorganic nanoparticles.

3.1. Gene delivery by physical methods

The simplest approach to non-viral delivery systems is direct gene transfer with naked plasmid DNA, which is not complexed with any carrier. Naked DNA is delivered directly to the cytoplasm, bypassing endosomes and lysosomes, and hence avoiding enzymatic degradation. However, naked DNA is degraded rapidly by cellular nucleases and cleared by the mononuclear-phagocyte system. In addition, DNA injected directly into a tissue drains rapidly into the lymphatic, and endonucleases in the extracellular space can rapidly degrade non-viral DNA. Several approaches have been developed to enhance the efficiency of gene transfer *via* naked DNA including gene gun and electroporation.

Gene gun is an ideal method for direct delivery of the gene into skin, mucosa, or surgically exposed tissues within a confined area [22]. Shooting gold particles coated with DNA allows direct penetration through the cell membrane into cytoplasm and the nucleus. However, the application of gene gun has results in short-term and low level expression of the gene product. Further improvements could include chemical modification of the surface of the gold particles to allow higher capacity and better consistency for DNA coating, and fine-tuning of the expelling force for precise control of DNA deposition into cells in various tissues [23].

Electroporation is a well established method for delivery of DNA and other molecules into cells *in vivo* and *in vitro*. This approach should work for any tissues into which a pair of electrodes can be inserted [24]. This method can achieve long-term expression after a single electroporation treatment. However, this application has several drawbacks. It has a limited effective range between the electrodes, and a surgical procedure is required. High voltage applied to tissues can also result in irreversible damage to them as a result of thermal heating [25].

Non-viral GDSs lack mechanisms for integration into the host chromosome. For this reason, combination of DNA transposon-based vectors is required for gene delivery. DNA transposons are natural genetic elements residing in the genome as repetitive sequences that move through a direct cut-and-paste mechanism. A simple transposon is organized by terminal inverted repeats embracing a gene encoding transposes, an enzyme required for its relocation [26]. DNA transposons have the desired features possessed by naked DNA and plasmids as well as the ability to insert transgenes into host chromosomes for long-term transgene expression [26].

3.2. Gene delivery by chemical methods

3.2.1. Cationic polymer-based GDSs

Cationic polymers can form stable complexes with genetic materials through electrostatic interactions under physiologic conditions. Commonly used cationic polymers are polyethylenimine (PEI), poly-L-lysine (PLL), chitosan, and polyamidoamines (PAMAM). The charges of cationic polymers used for forming an electrostatic complex with DNA come from primary, secondary, tertiary, and quaternary amino groups. For example, branched PEI possesses primary, secondary, and tertiary amino groups, while linear PEI has mostly secondary amines. PLL and its derivatives have primary amines, chitosan and its derivatives have primary or modified quaternary amino groups. The following strategies are used to develop cationic complexes with efficient cellular uptake: (1) *Cationization of complexes* - the higher the positive charge is on the surface of the complexes [27], the higher the affinity is for the negatively charged membrane, thereby resulting in a higher rate of uptake. However, highly positive surface charges induce cellular toxicity. (2) *The use of viral protein transduction domains (PTDs)* - PTDs mediate the entry of large biomolecules directly into the cytoplasm without endocytotic mechanisms. Some PTDs even promote transport across the nuclear envelope [28]. (3) *Targeting* - Conjugating target moieties to the complexes may induce receptor-mediated endocytosis. Some reported target moieties are fibronectin [29] or kistrin that bind to integrins [30], transferrin that binds to transferrin receptor [31], saccharide ligands that bind to asialoglycoprotein receptor (ASGPr) [32], antibodies that bind to their target structures, and growth factors that bind to growth factor receptors [33].

3.2.1.1. Polyethylenimine (PEI)-based polyplexes

Since polyethylenimine (PEI) was introduced for GDS by Behr in 1995 [4], it has been one of the most widely used GDS materials. PEIs have a high charge density, which offers higher transfection efficiency and higher protection against nuclease degradation than other cationic polymers. However, toxicity due to the high amount of positive charge and the resistance to biodegradation are disadvantages for the use of PEI as GDS *in vivo* [34]. It is known that the toxicity and transfection activity of PEI are dependent on molecular weight and structure [35]. An optimum molecular weight seems to range between 11.9 and 70 kDa [35]. Although both linear and branched PEIs have excellent transfection activities *in vitro*, linear PEI is less toxic than branched PEI when added to cells [25]. A large variety of PEI-based complexes have been developed to facilitate transfection with lower cytotoxicity. A folate-grafted PEI-600 cyclodextrin is an effective polyplex-forming plasmid delivery agent with low toxicity [36]. PEI-PEG polyelectrolyte complex (PEC) micelles were used for delivery of siRNA into tissue cells in tumor-bearing mice [37]. Polyester amine (PEA) copolymer based on poly-L-lactic and low molecular weight PEI were delivered into HEK293 cells [38], and PEI-PEG complexed with Ala-Pro-Arg-Pro-Gly peptide (APRPG) and siRNA conjugates were used for anti-angiogenic vascular endothelial growth factor tumor-targeted therapy [39].

3.2.1.2. Poly-L-lysine (PLL)-based polyplexes

Poly-L-lysines (PLLs) were developed earlier than other cationic polymers utilized for GDS, and a large variety of polymers with different molecular weights have been utilized in physiochemical and biological experiments. Due to their peptide structures, PLLs are biodegradable, which makes them suitable for use *in vivo*, however, PLLs show modest to high toxicity. The efficiency of cellular uptake of the PLLs is similar to that of PEIs, however, transfection efficiency is much lower, because PLLs less efficiently rupture endosomes. In order to increase transfection efficiency, several conjugates such as endosomolytic agents, histidine, and imidiazole have been used to develop PLL-based GDS [40].

3.2.1.3. Chitosan-based polyplexes

Chitosan is a natural polysaccharide that is extracted from crustaceans such as crabs and shrimp. Chitosan was developed as a GDS because it is non-toxic, biocompatible, and biodegradable. The Chitosan-based carrier has been reported for a variety of applications that include immunization, delivery of peptides and proteins. Huh *et al*. (2010) designed a new nano-sized siRNA carrier system composed of amphiphilic glycol chitosan (GC) and strongly positively charged PEI [41]. Ji *et al*. (2009) reported that chitosan/siRNA nanoparticles downregulated 66.9% of FHL2 gene expression *in vitro* [42]. Talaei *et al*. transfected doxorubicin (DOX) and antisense oligonucleotide (ASOND)-loaded on thiolated chitosan polymers into T47D breast cancer cells *in vitro* [43]. Several clinical trials are ongoing using chitosan-based systems, which will provide safety information in humans in the near future.

3.2.1.4. Dendrimer-based polyplexes

Dendrimers are multivalent synthetic macromolecules based on a well-defined cascade motif with a spherical shape as well as defect-free and perfectly monodisperse characteristics. Dendrimers have three typical structural properties; a central core, repeated branches and terminal functional groups. The repeated degree of branching occurs in the generation. The 6-generation PAMAMs are common dendrimers used for genetic transfection. They show high densities of amines

in the periphery of the molecule and show significantly enhanced levels of reporter gene expression compared to the intact polymer. Electrostatic interactions between the anionic phosphate groups of the DNA backbone and positively charged PAMAM dendrimers result in the formation of nano-scale complexes that prevent the degradation of DNA. Polyglycerol (PG)-based dendrimer core shell structures exhibiting low cytotoxicity have been developed to deliver siRNA to tumors *in vivo* [44].

3.2.2. Lipid-based complexes

Lipid-based complexes are cationic lipids, liposomes and micelles, which have been used in the last few decades as GDSs. Lipid-based complexes interact with negatively charged nucleic acids through electrostatic interactions to form lipoplexes (lipid complexes). Genes can either be entrapped within a lipid core [45] or attached to the surface of the cationic lipid materials [46]. Negatively charged DNA cannot externally bind to neutral and ionic liposomes. For this reason, the sizes of the desired genes are limited because the genes need to be encapsulated. Previous studies reported that siRNA molecules entrapped within a lipid core are protected from enzymatic degradation in serum, thereby increasing their stability.

Lipid nanoparticles can be divided into two categories, cationic lipids and neutral liposomes [47]. Liposomes entrapping genes have a neutral surface charge, thus reducing cellular toxicity [48].The neutral charge of the liposomes hinders transfection because of the difficulty of passing the cellular barrier. However, cationic liposomes can form stable complexes with DNA fragments [49]. Commonly used cationic liposomes include zwitterionic lipid, the cationic lipid, dioleoyl phosphatidyl ethanoamines (DOPE) [50], 1,2-dioleoyl-3-trimethyl ammonium-propane (DOTAP) [51], and the stable nucleic acid liposome nanoparticles (SNALP) [52].

A magnetic nanoparticle formulation termed LipoMag consists of an oleic acid-coated magnetic nanocrystal core and a cationic lipid shell [53]. Compared to the commercially available PolyMag, this LipoMag displayed more efficient gene silencing in 9 of 13 cell lines tested, and better anti-tumor effects for gastric tumor-mice *in vivo*. By delivering a silencing RNA that targets the epidermal growth factor receptor of tumor vessels, tumor growth was inhibited with no evident adverse immune reaction or unwanted side effects [53].

Liposomes have the disadvantage of significant cytotoxicity [54]. Liposome/DNA complexes can lead to cell concentration, mitotic inhibition, and cytoplasmic vacuole formation. Cationic liposomes can have anti-proliferative activities and specific cytotoxicity in cells that lack proteoglycans [55]. It has also been reported that cationic liposomes may result in hepatic necrosis [56] and pulmonary toxicity [57]. Inflammatory reactions caused by liposomes are another disadvantage [58].

3.2.3. Inorganic nanoparticles for gene delivery systems

3.2.3.1. Iron oxide nanoparticles

Iron oxide nanoparticles (IONPs), superparamagnetic material sized between approximately 1 and 100 nm, have a long history of investigation. They have shown outstanding potential in biomedical research, including magnetic resonance imaging (MRI) contrast enhancement, drug delivery, hyperthermia, and cell separation/labeling [59]. IONPs have advantageous properties for *in vivo* molecular imaging and delivery of drugs and genes; (1) a magnetic property such as providing an MR-based read-out of magnetically manipulated and charged particles, (2) compatibility such as biodegradation, metabolism, and integration into the serum Fe pool to form hemoglobin or to enter other metabolic processes, (3) a chemical property such as a large surface area for carrying drugs and genes.

In order to produce IONPs that are highly efficient for gene delivery, the IONPs should have an enhanced cationic surface with cationic polymers such as PEI, PLL, and chitosan. Various PEI-coated IONPs have been reported for *in vitro* non-viral gene delivery [60], however, these PEI-IONPs are limited for *in vivo* applications due to cellular toxicity. In order to overcome cellular toxicity, various polymers have been utilized to coat or conjugate to the IONPs. As an example, Chen *et al*. conjugated a T cell-specific ligand to PEGylated PEI-stabilized IONPs, which led to gene transfection enhancement with low cytotoxicity [61].

3.2.3.2. Carbon nanotubes

Carbon nanotubes (CNTs) are composed of one-dimensional seamless cylindrical grapheme sheets. They are divided into single-walled carbon nanotubes (SWNTs) composed of a single grapheme sheet and multi-walled carbon nanotubes (MWNTs) made of multiple concentric SWNTs. The diameters of SWNTs are around 1 nm and those of MWNTs are around 100 nm. Carbon nanotubes have been extensively studied for biomedical applications since 1991 [62]. Insoluble CNTs can be used as a GDS when they are chemically modified. They can be either covalently functionalized by oxidation and subsequent 1, 3-dipolar cyclo-addition reaction or non-covalently functionalized with hydrophobic or π-π stacking between the CNT and another non-polar ring such as the backbone of DNA. Amino-functionalized multi-walled carbon nanotubes (MWNT-NH_3^+) / siRNA complexes that have been administered intratumorally can elicit delayed tumor growth and increased survival of xenograft-bearing animals [63]. Recently, PEG-modified CNTs were

successfully tested in preclinical tests in the fields of oncology, neurology, vaccination, and imaging, suggesting that they are well-suited for the generation of novel multifunctional nano-drugs [64]. *In vitro* and *in vivo* studies have suggested that PEG-modified CNTs have favorable pharmacokinetic and toxicology profiles.

3.2.3.3. Silica nanoparticles

Silica nanoparticles (SiNPs) have been used as drug and gene delivery agents because they can be easily modified. Mesoporous silica nanoparticles (MSNPs) [65] and hollow silica nanoparticles (HSNPs) [66] are used for GDS and nano-drug carriers. SiNPs need to be modified with an anchoring group and charge transfer functional group to allow for DNA binding by electrostatic interactions for efficient cellular delivery. PLL is bound to SiNPs by electrostatic interactions to bind antisense DNA oligonucleotides and to enhance the endocytotic cellular uptake of the genetic material [67]. The regular arrangement of pores or hollow cavities in the silica nanoparticles easily accommodates siRNA molecules. Silica materials are usually toxic, but their toxicity may be reduced through surface modifications. For example, silica particles surface-coated with PEI become positively charged. These modified silica particles can pass through the cell membrane and enter the cytoplasm more easily with significantly reduced toxicity [68]. Lee *et al*. (2011) showed that PEGylated PEI-grafted silica nanoparticle-siRNA complexes enhance cellular uptake and efficient siRNA delivery with low cytotoxicity [69].

3.2.3.4. Gold nanoparticles

Gold nanoparticles (AuNPs) consist of colloidal gold suspended in liquids, sized from 1 to 150 nm. The sizes of gold nanoparticles can be determined by synthetic methods and capping agents. There are at least 3 synthetic methods according to reduction agents and related capping agents; (1) Reduction of AuCl (PPh3) with diborane or sodium borohydride makes 1 ~ 2 nm of core sized gold nanoparticles capped in phosphine [70]; (2) Two-phase liquid-liquid reduction of $HAuCl_4$ by sodiumborohydride in the presence of the thiol capping agent leads to gold nanoparticles with diameters of 1.5 ~ 5 nm [71]; (3) The most widely used synthesis method for gold nanoparticles involves reduction of chloroauric acid ($H[AuCl_4]$) with sodium citrate in water, which produce 10 ~ 150 nm of core-sized gold nanoparticles capped in aqueous citrate capping solution. Gold nanoparticles used for GDS are produced in a liquid by reduction of chloroauric acid, involving a two-phase process or the single phase water-based reduction of a gold salt by citrate. The gold nanoparticle concentrations are measured by UV-VIS spectroscopy with some coefficients, and size distribution and surface morphology can be obtained from transmission electron microscopy (TEM) or atomic force microscopy (AFM).

Gold nanoparticles are expected to be safe or have low toxicity; however, they have been described as both nontoxic [72] and toxic [73]. Cellular toxicity of AuNPs is dependent on core size and shape, and the material used for surface coating. Pan *et al*. evaluated the toxicity of AuNPs stabilized by triphenylphosphine derivatives (TPPMS and TPPTS) ranging in size from 0.8 to 15 nm in four cell lines such as, fibroblasts, epithelial cells, macrophages, and melanoma cells and found that 1.4 nm Au-TPPMS and Au-TPPTS of 1.4 nm were toxic with IC_{50} values of 46 and 30 μM, respectively [74]. In contrast, 15 nm Au-TPPMS was completely nontoxic up to 6,300 μM. Goodman *et al*. tested the cytotoxicity of cationic and anionic AuNPs for concentrations up to 0.38 ~ 3 μM in multiple cell lines and found that the cationic nanoparticles were clearly more cytotoxic than the anionic AuNPs [75].

Gold nanoparticles have emerged as an attractive and widely used nanomaterial for GDSs because they are inert and essentially nontoxic to cells. Furthermore, AuNPs can be easily functionalized by anchoring thiol linkers in their monolayers. Conjugate materials used for facilitating cellular uptake include peptides, proteins, antibodies, oligosaccharides, and nucleic acids [76]. Mirkin *et al*. (1996) developed AuNPs chemically functionalized with alkylthiol-terminated oligonucleotides [76]. These antisense oligodeoxynucleotide (ASODN)-modified nanoparticles have affinity constants for complementary nucleic acids that are higher than their unmodified oligonucleotide counterparts. Furthermore, AuNP-ASODNs are less susceptible to degradation by nuclease activity, exhibit greater than 99% cellular uptake, and are less toxic to the cells under the studied conditions [77].

Most gold nanoparticle bioconjugates are easily taken up by the cellular endocytotic mechanisms, but they remain trapped in endosomal vesicles and are incapable of being released into the cytosol. Endocytotic uptake efficiency of the gold nanoparticles is dependent on the nanoparticle surface chemistry and the physical properties (size and shape) of the material. Lysosomotropic agents, such as chloroquine or sucrose can be used to improve gene delivery and subsequent exogenous gene expression. Chloroquine is known to induce vesicular disruption by elevating the intravesicular pH of lysosomes and endosomes and increasing the availability and stability of AuNPs inside the cells [78].

A variety of functionalized AuNPs complexes conjugated with cell-penetrating peptides (CPPs), PEI, liposomes, or transferrin, have been developed as GDSs. Guo *et al*. (2010) reported that AuNPs coated with charge-reversal polyelectrolyte can be used for siRNA delivery [79]. Charge-reversal copolymers are often used to improve gene delivery efficiency by enhancing endosome escape capacity. They

demonstrated that after the surface of PEI/PAH-Cit/PEI/AuNPs was coated with a layer of polyelectrolyte bearing PEG and ligand moieties, the blood circulation time of PEI/PAH-Cit/PEI/AuNPs and the bioavailability of drugs improved [79]. Kong *et al*. (2011) reported that cationic lipid-coated AuNPs (L-AuNPs)-siRNA showed significant dose-dependent inhibition of GFP expression with low cytotoxicity for the MDA-MB-435 and A549 cells [80].

Kim *et al*. (2010) developed a functionalized gold nanoparticle-assisted universal carrier for antisense DNA [81], and showed that gold nanoparticles functionalized by single-stranded DNA can be used as a GDS without affecting normal cell physiology. Furthermore, applications of this system may be easily expandable to the delivery of siRNA, ribozyme, DNAzyme, and peptide-coding nucleotide acids [82,83]. The AuNP GDS-antisense conjugates efficiently knocked down the expression of target proteins and remained active for a longer period of time than those delivered by liposome formation. Although the delivery mechanism of AuNP conjugates is unknown, it has been hypothesized that proteins present at high concentrations in culture media may coat AuNP conjugates and consequently helps to deliver AuNPs into cells [84]. Consistent with this speculation, the gold nanoparticles appeared to gain a higher binding affinity to the cytoplasmic membrane of human cells when conjugated with DNA oligos [85].

4. Conclusion

Numerous gene delivery systems have been developed during the past decade. Despite high uptake efficiency, the use of most viral vectors is limited by the induction of host immune responses and the mutagenic integration of the viral genome into the host. Alternatively, non-viral systems are clearly non-pathogenic, but the cellular uptake efficiency is relatively low. In addition, the induction of inflammatory toxicity and the rapid clearance of non-viral systems are barriers for the development of successful gene delivery systems. Considering the importance of efficient and safe delivery of nucleic acids into living systems for many research areas including basic biology and medical applications, ongoing efforts will certainly provide circumstances to develop new and/or improved methods that can circumvent the disadvantages of current gene delivery systems.

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References

1. Gardlik, R., R. Palffy, J. Hodosy, J. Lukacs, J. Turna, and P. Celec (2005) Vectors and delivery systems in gene therapy. *Med. Sci. Monit*. 11: 110-121.
2. Lundstrom, K. and T. Boulikas (2003) Viral and non-viral vectors in gene therapy: Technology development and clinical trials. *Technol. Cancer Res. Treat*. 2: 471-486.
3. Mukherjee, S., R. N. Ghosh, and F. R. Maxfield (1997) Endocytosis. *Physiol. Rev*. 77: 759-803.
4. Boussif, O., F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix, and J. P. Behr (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: Polyethylenimine. *Proc. Natl. Acad. Sci. USA*. 92: 7297-7301.
5. Friend, D. S., D. Papahadjopoulos, and R. J. Debs (1996) Endocytosis and intracellular processing accompanying transfection mediated by cationic liposomes. *Biochim. Biophys. Acta* 1278: 41-50.
6. El Ouahabi, A., M. Thiry, V. Pector, R. Fuks, J. M. Ruysschaert, and M. Vandenbranden (1997) The role of endosome destabilizing activity in the gene transfer process mediated by cationic lipids. *FEBS Lett*. 414: 187-192.
7. Prasad, T. K. and N. M. Rao (2005) The role of plasmid constructs containing the SV40 DNA nuclear-targeting sequence in cationic lipid-mediated DNA delivery. *Cell Mol. Biol. Lett*. 10: 203-215.
8. Rosenberg, S. A., P. Aebersold, K. Cornetta, A. Kasid, R. A. Morgan, R. Moen, E. M. Karson, M. T. Lotze, J. C. Yang, and S. L. Topalian1990) Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N. Engl. J. Med*. 323: 570-578.
9. Yu, S. F., T. von Ruden, P. W. Kantoff, C. Garber, M. Seiberg, U. Ruther, W. F. Anderson, E. F. Wagner, and E. Gilboa (1986) Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells. *Proc. Natl. Acad. Sci. USA*. 83: 3194-3198.
10. Hacein-Bey-Abina, S., F. Le Deist, F. Carlier, C. Bouneaud, C. Hue, J. P. De Villartay, A. J. Thrasher, N. Wulffraat, R. Sorensen, S. Dupuis-Girod, A. Fischer, E. G. Davies, W. Kuis, L. Leiva, and M. Cavazzana-Calvo (2002) Sustained correction of X-linked severe combined immunodeficiency by *ex vivo* gene therapy. *N Engl. J. Med*. 346: 1185-1193.
11. Rainov, N. G. and H. Ren (2003) Clinical trials with retrovirus mediated gene therapy--what have we learned? *J. Neurooncol*. 65: 227-236.
12. Weinberg, J. B., T. J. Matthews, B. R. Cullen, and M. H. Malim (1991) Productive human immunodeficiency virus type 1 (HIV-1) infection of nonproliferating human monocytes. *J. Exp. Med*. 174: 1477-1482.
13. Follenzi, A., G. Sabatino, A. Lombardo, C. Boccaccio, and L. Naldini (2002) Efficient gene delivery and targeted expression to hepatocytes *in vivo* by improved lentiviral vectors. *Hum. Gene Ther*. 13: 243-260.
14. Bett, A. J., W. Haddara, L. Prevec, and F. L. Graham (1994) An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. *Proc. Natl. Acad. Sci. USA*. 91: 8802-8806.
15. Yang, Y., K. U. Jooss, Q. Su, H. C. Ertl, and J. M. Wilson (1996) Immune responses to viral antigens versus transgene product in the elimination of recombinant adenovirus-infected hepatocytes *in vivo*. *Gene Ther*. 3: 137-144.
16. Chia, M. C., W. Shi, J. H. Li, O. Sanchez, C. A. Strathdee, D. Huang, P. Busson, H. J. Klamut, and F. F. Liu (2004) A conditionally replicating adenovirus for nasopharyngeal carcinoma

gene therapy. *Mol. Ther*. 9: 804-817.
17. Murphy, J. E., S. Zhou, K. Giese, L. T. Williams, J. A. Escobedo, and V. J. Dwarki (1997) Long-term correction of obesity and diabetes in genetically obese mice by a single intramuscular injection of recombinant adeno-associated virus encoding mouse leptin. *Proc. Natl. Acad. Sci. USA*. 94: 13921-13926.
18. Wright, J. F., G. Qu, C. Tang, and J. M. Sommer (2003) Recombinant adeno-associated virus: formulation challenges and strategies for a gene therapy vector. *Curr. Opin. Drug. Discov. Devel*. 6: 174-178.
19. Wendtner, C. M., D. M. Kofler, H. D. Theiss, C. Kurzeder, R. Buhmann, C. Schweighofer, L. Perabo, S. Danhauser-Riedl, J. Baumert, W. Hiddemann, M. Hallek, and H. Buning (2002) Efficient gene transfer of CD40 ligand into primary B-CLL cells using recombinant adeno-associated virus (rAAV) vectors. *Blood* 100: 1655-1661.
20. Manservigi, R., R. Argnani, and P. Marconi (2010) HSV Recombinant Vectors for Gene Therapy. *Open Virol. J*. 4: 123-156.
21. Carpenter, D. E. and J. G. Stevens (1996) Long-term expression of a foreign gene from a unique position in the latent herpes simplex virus genome. *Hum. Gene Ther*. 7: 1447-1454.
22. Yang, N. S., J. Burkholder, B. Roberts, B. Martinell, and D. McCabe (1990) *In vivo* and in vitro gene transfer to mammalian somatic cells by particle bombardment. *Proc. Natl. Acad. Sci. USA*. 87: 9568-9572.
23. O'Brien, J. and S. C. Lummis (2002) An improved method of preparing microcarriers for biolistic transfection. *Brain Res. Brain Res. Protoc*. 10: 12-15.
24. Heller, L. C., K. Ugen, and R. Heller (2005) Electroporation for targeted gene transfer. *Expert. Opin. Drug Deliv*. 2: 255-268.
25. Gao, X., K. S. Kim, and D. Liu (2007) Nonviral gene delivery: What we know and what is next. *Aaps. J*. 9: 92-104.
26. Meir, Y. J. and S. C. Wu (2011) Transposon-based vector systems for gene therapy clinical trials: challenges and considerations. *Chang. Gung. Med. J*. 34: 565-579.
27. Blau, S., T. T. Jubeh, S. M. Haupt, and A. Rubinstein (2000) Drug targeting by surface cationization. *Crit. Rev. Ther. Drug Carrier Syst*. 17: 425-465.
28. Frankel, A. D. and C. O. Pabo (1988) Cellular uptake of the tat protein from human immunodeficiency virus. *Cell*. 55: 1189-1193.
29. Pierschbacher, M. D. and E. Ruoslahti (1984) Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* 309: 30-33.
30. Hart, S. L., R. P. Harbottle, R. Cooper, A. Miller, R. Williamson, and C. Coutelle (1995) Gene delivery and expression mediated by an integrin-binding peptide. *Gene Ther*. 2: 552-554.
31. Kircheis, R., A. Kichler, G. Wallner, M. Kursa, M. Ogris, T. Felzmann, M. Buchberger, and E. Wagner (1997) Coupling of cell-binding ligands to polyethylenimine for targeted gene delivery. *Gene Ther*. 4: 409-418.
32. Hashida, M., S. Takemura, M. Nishikawa, and Y. Takakura (1998) Targeted delivery of plasmid DNA complexed with galactosylated poly(L-lysine). *J. Control. Rel*. 53: 301-310.
33. Blessing, T., M. Kursa, R. Holzhauser, R. Kircheis, and E. Wagner (2001) Different strategies for formation of pegylated EGF-conjugated PEI/DNA complexes for targeted gene delivery. *Bioconjug. Chem*. 12: 529-537.
34. Fischer, D., T. Bieber, Y. Li, H. P. Elsasser, and T. Kissel (1999) A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: Effect of molecular weight on transfection efficiency and cytotoxicity. *Pharm. Res*. 16: 1273-1279.
35. Fischer, D., Y. Li, B. Ahlemeyer, J. Krieglstein, and T. Kissel (2003) *In vitro* cytotoxicity testing of polycations: Influence of polymer structure on cell viability and hemolysis. *Biomat*. 24: 1121-1131.
36. Yao, H., S. S. Ng, W. O. Tucker, Y. K. Tsang, K. Man, X. M. Wang, B. K. Chow, H. F. Kung, G. P. Tang, and M. C. Lin (2009) The gene transfection efficiency of a folate-PEI600-cyclodextrin nanopolymer. *Biomat*. 30: 5793-5803.
37. Lu, Z. X., L. T. Liu, and X. R. Qi (2011) Development of small interfering RNA delivery system using PEI-PEG-APRPG polymer for antiangiogenic vascular endothelial growth factor tumor-targeted therapy. *Int. J. Nanomed*. 6: 1661-1673.
38. Guo, Q. F., T. T. Liu, X. Yan, X. H. Wang, S. Shi, F. Luo, and Z. Y. Qian (2011) Synthesis and properties of a novel biodegradable poly(ester amine) copolymer based on poly(L-lactide) and low molecular weight polyethylenimine for gene delivery. *Int. J. Nanomed*. 6: 1641-1649.
39. Zong, C., K. Ai, G. Zhang, H. Li, and L. Lu (2011) Dual-emission fluorescent silica nanoparticle-based probe for ultrasensitive detection of $Cu2^{+}$. *Anal. Chem*. 83: 3126-3132.
40. Midoux, P. and M. Monsigny (1999) Efficient gene transfer by histidylated polylysine/pDNA complexes. *Bioconjug. Chem*. 10: 406-411.
41. Huh, M. S., S. Y. Lee, S. Park, S. Lee, H. Chung, Y. Choi, Y. K. Oh, J. H. Park, S. Y. Jeong, K. Choi, K. Kim, and I. C. Kwon (2010) Tumor-homing glycol chitosan/polyethylenimine nanoparticles for the systemic delivery of siRNA in tumor-bearing mice. *J. Control. Rel*. 144: 134-143.
42. Ji, A. M., D. Su, O. Che, W. S. Li, L. Sun, Z. Y. Zhang, B. Yang, and F. Xu (2009) Functional gene silencing mediated by chitosan/siRNA nanocomplexes. *Nanotechnol.* 20: 405103.
43. Talaei, F., E. Azizi, R. Dinarvand, and F. Atyabi (2011) Thiolated chitosan nanoparticles as a delivery system for antisense therapy: Evaluation against EGFR in T47D breast cancer cells. *Int. J. Nanomed*. 6: 1963-1975.
44. Ofek, P., W. Fischer, M. Calderon, R. Haag, and R. Satchi-Fainaro (2010) *In vivo* delivery of small interfering RNA to tumors and their vasculature by novel dendritic nanocarriers. *FASEB J.* 24: 3122-3134.
45. Li, J., Y. C. Chen, Y. C. Tseng, S. Mozumdar, and L. Huang (2010) Biodegradable calcium phosphate nanoparticle with lipid coating for systemic siRNA delivery. *J. Control. Rel*. 142: 416-421.
46. Barichello, J. M., T. Ishida, and H. Kiwada (2010) Complexation of siRNA and pDNA with cationic liposomes: The important aspects in lipoplex preparation. *Methods Mol. Biol*. 605: 461-472.
47. Semple, S. C., A. Akinc, J. X. Chen, A. P. Sandhu, B. L. Mui, C. K. Cho, D. W. Y. Sah, D. Stebbing, E. J. Crosley, E. Yaworski, I. M. Hafez, J. R. Dorkin, J. Qin, K. Lam, K. G. Rajeev, K. F. Wong, L. B. Jeffs, L. Nechev, M. L. Eisenhardt, M. Jayaraman, M. Kazem, M. A. Maier, M. Srinivasulu, M. J. Weinstein, Q. M. Chen, R. Alvarez, S. A. Barros, S. De, S. K. Klimuk, T. Borland, V. Kosovrasti, W. L. Cantley, Y. K. Tam, M. Manoharan, M. A. Ciufolini, M. A. Tracy, A. de Fougerolles, I. MacLachlan, P. R. Cullis, T. D. Madden, and M. J. Hope (2010) Rational design of cationic lipids for siRNA delivery. *Nat. Biotechnol*. 28: 172-118.
48. Spagnou, S., A. D. Miller, and M. Keller (2004) Lipidic carriers of siRNA: Differences in the formulation, cellular uptake, and delivery with plasmid DNA. *Biochem*. 43: 13348-13356.
49. Peer, D., E. J. Park, Y. Morishita, C. V. Carman, and M. Shimaoka (2008) Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target. *Sci*. 319: 627-630.
50. Guissouma, H., M. S. Froidevaux, Z. Hassani, and B. A. Demeneix (2006) *In vivo* siRNA delivery to the mouse hypothalamus confirms distinct roles of TR beta isoforms in regulating TRH transcription. *Neurosci. Lett*. 406: 240-243.
51. Taetz, S., A. Bochot, C. Surace, S. Arpicco, J. M. Renoir, U. F. Schaefer, V. Marsaud, S. Kerdine-Roemer, C. M. Lehr, and E. Fattal (2009) Hyaluronic acid-modified DOTAP/DOPE liposomes for the targeted delivery of anti-telomerase siRNA to

CD44-expressing lung cancer cells. *Oligonucleotides*. 19: 103-116.
52. Zimmermann, T. S., A. C. Lee, A. Akinc, B. Bramlage, D. Bumcrot, M. N. Fedoruk, J. Harborth, J. A. Heyes, L. B. Jeffs, M. John, A. D. Judge, K. Lam, K. McClintock, L. V. Nechev, L. R. Palmer, T. Racie, I. Rohl, S. Seiffert, S. Shanmugam, V. Sood, J. Soutschek, I. Toudjarska, A. J. Wheat, E. Yaworski, W. Zedalis, V. Koteliansky, M. Manoharan, H. P. Vornlocher, and I. MacLachlan (2006) RNAi-mediated gene silencing in non-human primates. *Nature* 441: 111-114.
53. Namiki, Y., T. Namiki, H. Yoshida, Y. Ishii, A. Tsubota, S. Koido, K. Nariai, M. Mitsunaga, S. Yanagisawa, H. Kashiwagi, Y. Mabashi, Y. Yumoto, S. Hoshina, K. Fujise, and N. Tada (2009) A novel magnetic crystal-lipid nanostructure for magnetically guided *in vivo* gene delivery. *Nat. Nanotechnol*. 4: 598-606.
54. Filion, M. C. and N. C. Phillips (1998) Major limitations in the use of cationic liposomes for DNA delivery. *Int. J. Pharm*. 162: 159-170.
55. Belting, M. and P. Petersson (1999) Protective role for proteoglycans against cationic lipid cytotoxicity allowing optimal transfection efficiency *in vitro*. *Biochem J*. 342: 281-286.
56. Loisel, S., C. Le Gall, L. Doucet, C. Ferec, and V. Floch (2001) Contribution of plasmid DNA to hepatotoxicity after systemic administration of lipoplexes. *Hum. Gene Ther*. 12: 685-696.
57. Li, S., S. P. Wu, M. Whitmore, E. J. Loeffert, L. Wang, S. C. Watkins, B. R. Pitt, and L. Huang (1999) Effect of immune response on gene transfer to the lung *via* systemic administration of cationic lipidic vectors. *Am. J. Physiol*. 276: 796-804.
58. Dokka, S., D. Toledo, X. G. Shi, V. Castranova, and Y. Rojanasakul (2000) Oxygen radical-mediated pulmonary toxicity induced by some cationic liposomes. *Pharmaceut. Res*. 17: 521-525.
59. Dobson, J. (2006) Gene therapy progress and prospects: Magnetic nanoparticle-based gene delivery. *Gene Ther*. 13: 283-287.
60. Scherer, F., M. Anton, U. Schillinger, J. Henke, C. Bergemann, A. Kruger, B. Gansbacher, and C. Plank (2002) Magnetofection: Enhancing and targeting gene delivery by magnetic force *in vitro* and *in vivo*. *Gene Ther*. 9: 102-109.
61. Chen, G. H., W. J. Chen, Z. Wu, R. X. Yuan, H. Li, J. M. Gao, and X. T. Shuai (2009) MRI-visible polymeric vector bearing CD3 single chain antibody for gene delivery to T cells for immunosuppression. *Biomat*. 30: 1962-1970.
62. Iijima, S. (1991) Helical microtubules of graphitic carbon. *Nature* 354: 56-58.
63. Podesta, J. E., K. T. Al-Jamal, M. A. Herrero, B. Tian, H. Ali-Boucetta, V. Hegde, A. Bianco, M. Prato, and K. Kostarelos (2009) Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model. *Small*. 5: 1176-1185.
64. Bottini, M., N. Rosato, and N. Bottini (2011) PEG-modified carbon nanotubes in biomedicine: Current status and challenges ahead. *Biomacromol*. 12: 3381-3393.
65. Radu, D. R., C. Y. Lai, K. Jeftinija, E. W. Rowe, S. Jeftinija, and V. S. Lin (2004) A polyamidoamine dendrimer-capped mesoporous silica nanosphere-based gene transfection reagent. *J. Am. Chem. Soc*. 126: 13216-13217.
66. Yang, J., J. Lee, J. Kang, K. Lee, J. S. Suh, H. G. Yoon, Y. M. Huh, and S. Haam (2008) Hollow silica nanocontainers as drug delivery vehicles. *Langmuir*. 24: 3417-3421.
67. Zhu, S. G., J. J. Xiang, X. L. Li, S. R. Shen, H. B. Lu, J. Zhou, W. Xiong, B. C. Zhang, X. M. Nie, M. Zhou, K. Tang, and G. Y. Li (2004) Poly(L-lysine)-modified silica nanoparticles for the delivery of antisense oligonucleotides. *Biotechnol. Appl. Biochem*. 39: 179-187.
68. Xia, T., M. Kovochich, M. Liong, H. Meng, S. Kabehie, S. George, J. I. Zink, and A. E. Nel (2009) Polyethyleneimine coating enhances the cellular uptake of mesoporous silica nanoparticles and allows safe delivery of siRNA and DNA constructs. *ACS Nano*. 3: 3273-3286.
69. Lee, H., D. Sung, M. Veerapandian, K. Yun, and S. W. Seo (2011) PEGylated polyethyleneimine grafted silica nanoparticles: Enhanced cellular uptake and efficient siRNA delivery. *Anal. Bioanal. Chem*. 400: 535-545.
70. Schmid, G. (1992) Large clusters and colloids - metals in the embryonic state. *Chem. Rev*. 92: 1709-1727.
71. Brust, M., M. Walker, D. Bethell, D. J. Schiffrin, and R. Whyman (1994) Synthesis of Thiol-derivatized gold nanoparticles in a 2-phase liquid-liquid system. *J. Chem. Soc. Chem. Comm*. 801-802.
72. Connor, E. E., J. Mwamuka, A. Gole, C. J. Murphy, and M. D. Wyatt (2005) Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small*. 1: 325-327.
73. Goodman, C. M., C. D. McCusker, T. Yilmaz, and V. M. Rotello (2004) Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjug. Chem*. 15: 897-900.
74. Pan, Y., A. Leifert, D. Ruau, S. Neuss, J. Bornemann, G. Schmid, W. Brandau, U. Simon, and W. Jahnen-Dechent (2009) Gold nanoparticles of diameter 1.4 nm trigger necrosis by oxidative stress and mitochondrial damage. *Small*. 5: 2067-2076.
75. Kumar, S., J. Aaron, and K. Sokolov (2008) Directional conjugation of antibodies to nanoparticles for synthesis of multiplexed optical contrast agents with both delivery and targeting moieties. *Nat. Protoc*. 3: 314-320.
76. Mirkin, C. A., R. L. Letsinger, R. C. Mucic, and J. J. Storhoff (1996) A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* 382: 607-609.
77. Rosi, N. L., D. A. Giljohann, C. S. Thaxton, A. K. Lytton-Jean, M. S. Han, and C. A. Mirkin (2006) Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Sci*. 312: 1027-1030.
78. Sandhu, K. K., C. M. McIntosh, J. M. Simard, S. W. Smith, and V. M. Rotello (2002) Gold nanoparticle-mediated transfection of mammalian cells. *Bioconjug. Chem*. 13: 3-6.
79. Guo, S., Y. Huang, Q. Jiang, Y. Sun, L. Deng, Z. Liang, Q. Du, J. Xing, Y. Zhao, P. C. Wang, A. Dong, and X. J. Liang (2010) Enhanced gene delivery and siRNA silencing by gold nanoparticles coated with charge-reversal polyelectrolyte. *ACS Nano*. 4: 5505-5511.
80. Kong, W. H., K. H. Bae, S. D. Jo, J. S. Kim, and T. G. Park (2012) Cationic lipid-coated gold nanoparticles as efficient and non-cytotoxic intracellular siRNA delivery vehicles. *Pharmaceut. Res*. 29: 362-374.
81. Kim, J. H., H. H. Jang, S. M. Ryou, S. Kim, J. Bae, K. Lee, and M. S. Han (2010) A functionalized gold nanoparticles-assisted universal carrier for antisense DNA. *Chem. Commun. (Camb)*. 46: 4151-4153.
82. Ryou, S. M., S. Kim, H. H. Jang, J. H. Kim, J. H. Yeom, M. S. Eom, J. Bae, M. S. Han, and K. Lee (2010) Delivery of shRNA using gold nanoparticle-DNA oligonucleotide conjugates as a universal carrier. *Biochem. Biophys. Res. Commun*. 398: 542-546.
83. Ryou, S. M., M. Park, J. M. Kim, C. O. Jeon, C. H. Yun, S. H. Han, S. W. Kim, Y. Lee, S. Kim, M. S. Han, J. Bae, and K. Lee (2011) Inhibition of xenograft tumor growth in mice by gold nanoparticle-assisted delivery of short hairpin RNAs against Mcl-1L. *J. Biotechnol*. 156: 89-94.
84. Giljohann, D. A., D. S. Seferos, P. C. Patel, J. E. Millstone, N. L. Rosi, and C. A. Mirkin (2007) Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles. *Nano Lett*. 7: 3818-3821.
85. Kim, Y. J., S. M. Ryou, S. Kim, J. H. Yeom, M. S. Han, K. Lee, and M. J. Seong (2012) Enhanced protein-mediated binding between oligonucleotide–gold nanoparticle composites and cell surfaces: Co-transport of proteins and composites. *J Mater. Chem*. 22: 25036-25041.