Medicated Nanoparticle for Gene Delivery

Sakthivel Lakshmana Prabu, Timmadonu Narasimman Kuppusami Suriyaprakash and Rathinasabapathy Thirumurugan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65709

Abstract

Delivering the drug to the target site with a desired concentration to provide therapeutic effect is a major problem in the drug delivery system. Effectiveness, poor distribution and lack of selectivity are the drawbacks of the conventional dosage form. Recently Nanotechnology has been given much attention in various fields specifically in the biomedical application. Material includes organic, inorganic, polymeric and lipid-based nanobiomaterials after surface modification; it has been utilized for drug and gene delivery systems. Viral and non-viral vectors are the two types in gene delivery utilizing genetic materials like DNA plasmids, RNA and siRNA. Cellular and extracellular barriers are the two main barriers in gene delivery. The basic mechanism involved in the gene delivery is an introduction of a gene encoding a functional protein altering the expression of an endogenous gene or owning the capacity to cure or prevent the progression of a disease. Nanoparticle surface features like particle shape and surface charge are having major roles in the gene delivery. To provide the site-specific delivery various properties like nature of polymer, particle size, solubility, biocompatibility, biodegradability and nanoparticle surface features are need to be considered. Gene delivery has been utilized for various disease treatments such as cancer, AIDS, and cardiovascular diseases.

Keywords: Gene delivery, DNA, RNA, Nanoparticle
1. Introduction

Drug and gene delivery system include organic, inorganic, polymeric and lipid-based nanobiomaterials. Binding of the nanobiomaterials to the receptors to target cells/tissues can be improved by surface modification. This surface modification may increase solubility, immune compatibility, and cellular uptake.

Various nano drug delivery systems include nanoparticles, nanocapsules, nanotubes, nanogels, and dendrimers. They can be used to deliver both small molecule drugs and various classes of biomacromolecules, such as peptides, proteins, plasmid DNA, and synthetic oligodeoxynucleotides. Antisense oligonucleotide (AS-ODN) and small interfering RNA (siRNA) are shown as promise one in gene delivery and good therapeutic agents, but it can be used directly due to their limitations such as sequence size, length, charge, half-life, or stability in solutions [1].

Various diseases are occurred in human beings due to mutations or deletions in genes lead to metabolic pathway disorder, regulation of cell cycle, protein function and its structure, function of receptor, and cell skeleton [2]. This can be treated effectively through gene delivery system. Gene delivery is a term used when referring to the delivery of genetic material such as DNA plasmids, RNA, and siRNA into target cells either encapsulated inside or conjugated to the NPs to express or suppress the biosynthesis of proteins (also called transfection) to treat or cure many diseases [3–10].

2. Various gene delivery mechanisms

2.1. Plasmid DNA

It is currently the most commonly investigated nucleic acid in gene delivery applications. When the pDNA is entering into the nucleus, the pDNA strand is transcribed, and the coding gene is translated to protein, which is then expressed from the cell.

2.2. RNA interference

It is triggered by double-stranded RNA (dsRNA), activates the anti-viral interferon leads to shutdown of protein synthesis by degradation of messenger RNA (mRNA). Another mechanism involves the use of microRNAs (miRNA), which are small non-coding nucleic acids responsible for post-translational regulation of protein expression.

2.3. Small interfering RNA

Small interfering RNA comprises around 21–23 nucleotides, which can be designed to be better targeted than long dsRNA and can eliminate the activation of the response of the interferon while still inhibiting target gene expression. The gene expression can be able to control/block transected siRNA into mammalian cells; this specific gene block can be used to treat certain infectious diseases and cancers [11–14].
To obtain an efficient vector system and to achieve a high rate of cell transfection, the following two limitations must be integrated in the development of an ideal genetic vector. In the gene transfer methods whether viral, physical, or chemical, these two major limitations must be overcome.

1. The first limitation is a carrier, which is needed to carry the nucleic acids to the target cells without potential risks. Naturally viruses having the ability to recognize and locate the defined target cells due to its body defense mechanisms, such as the reticulo-endothelial system (RES). Whereas the chemical vectors conjugate with targeting molecules to realize the specific location through various techniques.

2. The second limitation is the penetration of the nucleic acids into the cell through the plasma membrane. Viruses can achieve the same through natural mechanisms, whereas the chemical vectors must disturb the plasma membrane (e.g. physical vectors)/or internal vesicular membranes (e.g. the cationic lipids) [15].

3. Gene delivery

In gene delivery, a vector/carrier is essential in order to carry the hydrophilic, negatively charged DNA through the hydrophobic and negatively charged cell membrane. The therapeutic efficiency depends upon the efficient delivery of DNA into the target site. Barriers including cellular like intracellular uptake, endosomal escape, DNA release, and nuclear uptake and extracellular barriers like avoidance of particle clearance mechanisms, targeting to specific tissues and/or cells of interest, and protection of DNA from degradation are present in the system [16–19]. One main hurdle in gene delivery is the delivery of therapeutic poly-nucleotides crossing the plasma membrane and delivering into the cells of interest. This is the limitation one in the gene delivery for efficient and safe delivery into the cells. A good gene delivery vector should be able to effectively compact and protect DNA, sufficient stability during bypassing the immune system of the host, traverse the plasma membrane (typically through endocytosis), disrupt the endosomal membrane, and deliver the DNA into the nucleus [20–22]. Successful gene transfer requires sufficient stability of DNA during the extracellular delivery phase, transportation through cell membranes, cytoplasm, and eventual disassembly and nuclear delivery.

Gene delivery systems can be divided into two general categories:

1. Viral transduction systems

2. Nonviral transfection systems

Initially, viruses were used for gene delivery. The disadvantages of viral vectors limited their application in gene delivery like due to its size of DNA that they can carry, low loading capacity, large-scale manufacturing, quality control cost, and safety factor such as immunogenicity and potential oncogenicity [23].
Hence, more attention has been paid to develop non-viral vectors as an alternative one for gene delivery [6, 8–10, 24].

Nonviral delivery systems have advantages like easy to prepare, amenable to synthetic manipulations of polymer properties, cell/tissue targeting, less immunogenic and oncogenic, no potential of virus recombination and limitation on the size of a transferred gene, virtually no limitation on the unrestricted plasmid size that can be delivered and the cost of production is relatively low [25]. Moreover, they can be consigned readily to carry genetic materials to target cells by virtue of their size, charge and structurally modifying the vectors [26]. Difference between viral and nonviral gene delivery is based on the various gene transfer and its complementary mechanisms. The mechanism includes in the viral gene delivery is the ability of virus to circulate in the blood, bind to cell surface receptors, gain entry into the cell, avoid lysosomal destruction, survive degradation in the cytosol, and deliver genetic material to the nucleus. In the nonviral gene delivery overcoming biological barriers in the circulation or inside the target cell and transferring the gene vector is based on the molecular weight of the vector, ratio between the vector nitrogens and the DNA phosphates (termed the N:P ratio) and the salt concentration of the buffer solution. [27–30].

Nonviral gene delivery systems are typically composed of plasmid DNA condensed into nanoparticles by a cationic polymer [31].

Nonviral vectors are categories into lipid- and polymer-based one. Whereas the polymeric based nonviral vectors have the advantage over lipid-based one due to its modification property.

The steps involved in the polymeric gene delivery are given below:

- DNA/polymer complexation: Nanosize complex forms when cationic polymer neutralizes charged phosphate with negatively charged cell membrane.
- DNA/polymer complex: Also referred as polyplex, which passes through cell membrane by a nonspecific or receptor-mediated endocytosis.
- Endosome: Complex enters into cytoplasm through endosome.
- Transportation to nucleus.
- It is free to be encoded into a therapeutic protein or to be inserted into the genome [6, 8–10].

4. Targeted drug delivery

It is necessary to ensure that the nanomaterials are carefully delivered only to the infected region of the body without affecting the surrounding healthy tissues.

When drugs or gene-loaded nanoparticles are injected into bodies, they can circulate in the blood vessels by crossing the epithelial barriers before reaching the target site. Escape of nanoparticles from the vascular circulation occurs in either continuous or fenestrated tissues.
Nanoparticles can escape from the bloodstream at continuous vascular endothelium through paracellular pathway, intracellular process or transcellular pathway. It is different; the space between the fenestration sites on the endothelium is between 100 nm and 2 μm, which is longer than in healthy tissues that are normally 2–6 nm. Therefore, nanoparticles can penetrate fenestrations thus increase the drug concentration in target/tumor site which is called “enhanced permeation and retention effect (EPR effect)” [32–34]. Particle shape, surface charge, and feature are playing important roles in intercellular delivery [35, 36]. Quantity and type of polymers, particle size, solubility, biodegradability, and surface properties are having important role in release of bioactive drugs into the target site [37]. Drug entries through transcellular and paracellular pathways are shown in Figure 1.

![Figure 1](image1.png)

**Figure 1.** Drug entry through transcellular and paracellular pathways.

Targeted drug delivery is classified into two categories. They are

1. Passive targeting
2. Active targeting

### 4.1. Passive targeting

Passive targeting involves the cells that are to be targeted migrate toward the drug-carrying vehicles. This system is widely used in the delivery of cells like neutrophils, macrophages, dendritic cells for vaccination purposes. In this system, it is not necessary the drug-carrying vehicles in nanometer regime [38].

### 4.2. Active targeting

Active targeting involves rational design of nanosystems with suitable surface engineering performed with acceptable chemical linking strategies to specifically target the cell receptors of a target tissue. Furthermore, the targeting operates at two levels; first, the targeting of tissue/system in order to enrich the concentration of the carriers at the infected site [9, 39].
5. Nonviral vector gene delivery

Nonviral vector consists of either natural vectors (plasmid DNA or small nucleic acids, antisense oligonucleotides, small interfering RNAs) or synthetic vectors (liposomes, cationic polymers) [40]. Naked DNA, usually in plasmid form, is the simplest form of non-viral transferring of a gene into a target cell [41–44].

Nonviral vector delivery is categorized as organic (lipid complexes, conjugated polymers, cationic polymers, etc.) and inorganic systems (magnetic nanoparticles, quantum dots, carbon nanotubes, gold nanoparticles (GNPs), etc.) [45].

To achieve the desired therapeutic efficacy, a suitable carrier system is needed. Nanoparticles can be considered as a good carrier for various therapeutic applications due to the following reasons.

- They exist in the same size domain as proteins.
- They have large surface areas and ability to bind to a large number of surface functional groups.
- They possess controllable absorption and release properties and particle size and surface characteristics. [46].

6. Inorganic type nonviral delivery vectors

Inorganic type of nonviral delivery vectors are magnetic nanoparticles, quantum dots, and gold nanoparticles, and so on [31, 47].

6.1. Magnetic nanoparticle

Combination of inorganic nanoparticles with organic materials forms hybrids which possess unique physical, chemical, optical, and electrical properties. These unique properties can be utilized in different applications than large size materials. Recently, magnetic nanoparticles have been utilized as an effective tool in gene delivery because of its submicron size. Hence, much research has been carried out to control the size and shape of the metal nanostructure due to its magnetic, catalytic, electrical, and optical properties. Iron oxides, such as CoFe$_2$O$_4$, NiFe$_2$O$_4$ and MnFe$_2$O$_4$, exhibit superior performance compared to other magnetic materials but highly toxic to cells. The most widely used iron oxide as magnetic cores are magnetite (Fe$_3$O$_4$) and maghemite ($\gamma$-Fe$_2$O$_3$), possess high magnetic moments and relatively safe. The magnetic nanoparticle core is fairly reactive, prevents corrosion and leaking when applied in vivo. In the magnetic nanoparticle gene delivery system, the gene directly binds to the magnetic particle or carrier. In magnetic nanoparticle, a magnetic core is coated by a protective layer either by dispersing in a polymer matrix or encapsulated within a polymer/metallc shell, which can be combined with therapeutic agents (carrier/DNA complexes or other drugs) through covalent or noncovalent bond. Silica, gold, natural polymers, such as dextran, or...
synthetic polymers, such as PEI, PLL, PEG, and polyvinyl alcohol (PVA), are commonly used coating materials in magnetic nanoparticle. Introduction of various functional groups (organic linkers) like carboxyl, amines, thiols, and aldehyde can alter the surface properties to suit various therapeutic agents to improve targeted gene delivery. The preferred coating surface for magnetic particles is strongly cationic because of the negatively charged DNA molecules that are to be delivered. Magnetofection is a methodology based on the association of magnetic nanoparticle with gene vectors in order to optimize/enhance gene delivery in the presence of a magnetic field. The magnetic field is applied to move the MNP-gene vector complexes toward the target site. In magnetofection, gene can be delivered in few minutes to the target site, whereas traditional transfection methods can take several hours. Stability of any magnetic nanoparticles depends upon the balance between attractive (van der Waals and dipole-dipole) and repulsive (steric and electrostatic) forces between the particles and the surrounding solvent molecules. Temperature also has an effect in the stability of the magnetic nanoparticle due to energy transfer from the solvent molecules (Brownian motion) to the nanometric particles. Hence, magnetic nanoparticle can be coated with a biocompatible polymer to enhance its stability [30, 31, 48–62].

6.2. Metal nanoparticle (gold nanoparticle)

Owing to nano-dimension size to volume ratio and its stability, inorganic (metal) nanoparticles are being extensively used as promising gene carriers in various biomedical applications. Among the various metal nanoparticle gold nanoparticles (GNPs) are an obvious choice due to its inert, amenability of synthesis, high functionalization, fictionalization ability, higher absorption coefficient, good biocompatibility, less cytotoxic, ease of detection, and potential capability of targeted delivery, hence it is extensively used for various applications including drug and gene delivery. Due to its remarkable stability, large surface area, surface modification, and high biocompatibility, gold nanoparticles can retain the native structure and enzymatic activity of the attached proteins or enzymes in the drug delivery. Gold nanoparticles have large surface area due to which their surfaces are readily available for modification with targeting molecules or specific biomarkers and applicable in biomedical purposes.

Gold nanoparticles have large surface bio conjugation with molecular probes, and they also have many optical properties which are mainly concerned with localized plasmon resonance (PR). Gold nanoparticles can bind with a wide range of organic molecules and have tunable physical and chemical properties. Gold nanoparticles can be synthesized by chemical (seeding growth method), physical (γ-irradiation method, microwave irradiation method), and green methods (natural biomaterial egg shell membrane, sun light irradiation method).

Combination of gold nanoparticles into smart polymer like poly (N-isopropylacrylamine) is an effective process to enhance its properties. Gold nanoparticles exhibit different shapes such as spherical, sub-octahedral, octahedral, decahedral, icosahedral multiple twined, multiple twined, irregular shape, tetrahedral, nanotriangles, nanoprisms, hexagonal platelets, and nanorods, which are shown in Figure 2. Among the various shapes triangular-shaped nanoparticles show attractive optical properties compared with the spherical-shaped nanoparticles [30, 58, 63–72].
6.3. Quantum dots

Quantum dots are tiny semiconductor crystals of luminescent nanocrystals with rich surface chemistry and unique optical properties with the size of 1–10 nm made up of compounds from group II to VI and III to V, for example, Ag, Cd, Hg, Ln, P, Pb, Se, Te, Zn, and so on. QDs have distinctive characteristics such as size-tunable light emission, improved signal brightness, resistance against photobleaching, and simultaneous excitation of multiple fluorescence colors.

Depending on their size by laser, the quantum dots glow brightly in different colors, such as Adirondack Green (520nm), Blue (514 nm), Greenish blue (544 nm), Green (559 nm), Yellowish green (571 nm), Yellow (577 nm), Yellowish orange (581 nm), Fort Orange (600nm), Orange (610 nm), and Maple Red-Orange (620nm).

QDs are nearly spherical semiconductor particles with core-shell structure. Colloidal core/shell QDs, such as CdSe/ZnS, CdSe/CdS/ZnS, CdTe/CdSe, and InP/ZnS, are commonly synthesized for biomedical applications, whereas CdSe/ZnS, CdTe/ZnS, and CdSe/CdS/ZnS have been commonly used.

Quantum dots are made up of three parts, that is, core, shell, and cap.

Core is made up of CdSe, which is a semiconductor material. Core is surrounded by shell which is made up of ZnS for improving its optical properties and cap encapsulates the double layer quantum dots by different materials like silica which helps in improving solubility in aqueous buffers. Structure of quantum dot is shown in Figure 3.
The semiconducting nature and the size-dependent fluorescence of these nanocrystals have been successfully applied for in vitro, in vivo transfection and for diagnosis of various diseases. One of the most important emerging applications of QDs appears to be *traceable* drug delivery, because it has the potential to elucidate the pharmacokinetics and pharmacodynamics of drug candidates and to provide the design principles for drug carrier engineering.

In gene technology, the quantum dot can be conjugated with oligonucleotide sequences (attached via surface carboxylic acid groups) may be targeted to bind with DNA or mRNA. Gene-associated drugs can be loaded within a QD core or attached to the surface of these nanoparticles through direct conjugation or electrostatic complexation by which QDs can protect the gene from degradation by nucleases. This property has been utilized for an assay of single nucleotide polymorphism (SNP). Due to concerns about long-term *in vivo* toxicity and degradation, QDs are currently limited to cell and small animal uses [30, 31, 77–101].

### 7. Conclusion

Recently nanotechnology-based gene delivery is one of the most attractive therapeutic methods for treatment of various diseases. In drug delivery, size and distribution of particles are critical parameters to target specific organs and tissues. Proteins (derived from their secondary structure) are suitable materials for drug/gene carriers due to their precise molecular sizes. An ideal nanoparticle formulation for a drug or gene carrier system can achieve long circulation time, low immunogenicity, good biocompatibility, and selective targeting.

Gene delivery involves viral and non-viral vectors. Viral vectors are having low loading capacity, large-scale manufacturing, quality control cost, and safety factor such as immunogenicity and potential oncogenicity. From the stability and safety concern, non-viral vectors have more efficiently passing the gene transfection through the biological barriers compared to viral vectors. Organic, inorganic, and various hybrid materials are used for the preparation of nanoparticles. Among these, polymeric nanoparticles have great therapeutic application.
due to its wide range of sizes and varieties and can be used in sustained and targeted gene delivery for long periods. Biopolymers used for the preparation of nonviral vectors possess several favorable characteristics, such as high biocompatibility, low toxicity, good biodegradability, and abundant renewable sources, which can be used for efficiency delivery of drug/gene to the target site.

Choosing a suitable design of nanoparticle structure can increase gene transfection efficiency to overcome extracellular and intracellular transfection barriers: the blood stream, the cellular membrane, endosomes, and the nuclear membrane. Nanoparticle in gene delivery depends upon the nature of the polymer charge and its chain length. Furthermore, modifications in the nanoparticle by introducing ligands onto the surface can enhance localization and retention in specific target tissue, local delivery of agents to a large volume of tissues for better clinical application. However, biopolymer-based nanoparticle will become a tool in near future for the precisely targeted delivery of drugs and genes in many therapeutic fields, but toxicological issues and degradation products of nanoparticles are need to be considered before being applied into humans.

Author details

Sakthivel Lakshmana Prabu*, Timmadonu Narasimman Kuppusami Suriyaprakash and Rathinasabapathy Thirumurugan

*Address all correspondence to: slaxmanvel@gmail.com

1 Department of Pharmaceutical Technology, Bharathidasan Institute of Technology, Anna University, Tiruchirapalli, Tamilnadu, India

2 Dept of Pharmaceutics, Al Shifa College of Pharmacy, Kerala

3 School of Pharmacy, International Medical University, Malaysia

References

[1] Fattal E, Barratt G. Nanotechnologies and controlled release systems for delivery and antisense oligonucleotides and small interfering RNA. Br. J. Pharmacol. 2009; 157(2): 179–194.

[2] Erdal C, Ali DS, Emre SC. Gene delivery systems: recent progress in viral and non-viral therapy. In: Recent Advances in Novel Drug Carrier Systems, Ali DS, ed. Croatia, InTech, 2012. DOI: 10.5772/53392. http://www.intechopen.com/books/recent-advances-in-novel-drug-carrier-systems/gene-delivery-systems-recent-progress-in-viral-and-non-viral-therapy
[3] Kaul G, Amiji M. Tumor-targeted gene delivery using poly-(ethylene glycol)-modified gelatin nanoparticles: in vitro and in vivo studies. Pharm. Res. 2005; 22(6): 951–961.

[4] Durland RH, Eastman EM. Manufacturing and quality control of plasmid-based gene expression systems. Adv. Drug Deliv. Rev. 1998; 30: 33–48.

[5] Labhasetwar V, Chen B, Muller DWM, Bonadio J, Ciftci K, March K, et al. Gene-based therapies for restenosis. Adv. Drug Deliv. Rev. 1997; 24:109–20.

[6] Jordan M. Synthetic and semisynthetic polymers as vehicles for in vitro gene delivery into cultured mammalian cells. In: Synthetic Polymers for Biotechnology and Medicine, Freitag R, ed. Georgetown, DC: Eurekah.com/Landes Bioscience, 2003; pp. 19–39.

[7] Langer R. Introduction. In: Polymeric Gene Delivery: Principles and Applications, Amiji MM, ed. Boca Raton, FL: Taylor & Francis, 2005; pp. 1–2.

[8] Luten J, Van Nostruin CF, De Smedt SC, Hennink WE. Biodegradable polymers as nonviral carriers for plasmid DNA delivery. J Control. Release. 2008; 126(2): 97–110.

[9] Merdan T, Kopeczek J, Kissel T. Prospects for cationic polymers in gene and oligonucleotide therapy against cancer. Adv. Drug Deliv. Rev. 2002; 54(5): 715–758.

[10] Wong SY, Pelet JM, Putnam D. Polymer systems for gene delivery-past, present, and future. Prog. Pol. Sci. 2007; 32(8–9):799–837.

[11] Raftery R, O’Brien FJ, Cryan SA. Chitosan for gene delivery and orthopedic tissue engineering applications. Molecules. 2013; 18: 5611–5647.

[12] Rana TM. Illuminating the silence: Understanding the structure and function of small RNAs. Nat. Rev. Mol. Cell Biol. 2007; 8: 23–36.

[13] Guo P, Coban O, Snead NM, Trebley J, Hoeprich S, Guo S, et al. Engineering RNA for targeted siRNA delivery and medical application. Adv. Drug Deliv. Rev. 2010; 62: 650–666.

[14] Van Rooij E. The art of microRNA research. Circ. Res. 2011; 108: 219–234.

[15] Jin L, Zeng X, Liu M, Deng Y, He N. Current progress in gene delivery technology based on chemical methods and nano-carriers. Theranostics. 2014; 4(3): 240–255.

[16] Putnam D. Polymers for gene delivery across length scales. Nat. Mater. 2006; 5: 439–451.

[17] Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. Nat. Rev. Drug Discov. 2005; 4: 581–593.

[18] Mansouri S, Lavigne P, Corsi K, Benderdour M, Beaumont E, Fernandes JC. Chitosan-DNA nanoparticles as nonviral vectors in gene therapy: strategies to improve transfection efficacy. Eur. J Pharm. Biopharm. 2004; 57: 1–8.
[19] Yang Y, Li Q, Erte HCJ, Wilson JM. Cellular and humoral immune responses to viral antigens create barriers to lung directed gene therapy with recombinant adenoviruses. J Virol. 1995; 69: 2004–2015.

[20] Giannoukakis N, Thomson A, Robbins P. Gene therapy in transplantation. Gene Ther. 1999; 6(9): 1499–1511.

[21] Liu F, Liang KW, Huang L. Systemic administration of naked DNA: Gene transfer to skeletal muscle. Mol. Interv. 2001; 1(3): 168–172.

[22] Mahato RI. Non-viral peptide-based approaches to gene delivery. J. Drug Target. 1999; 7(4): 249–268.

[23] Itaka K, Kataoka K. Recent development of nonviral gene delivery systems with virus-like structures and mechanisms. Eur. J Pharm. Biopharm. 2009; 71: 475–483.

[24] Mrsny RJ. Tissue- and cell-specific targeting for the delivery of genetic information. In: Polymeric Gene Delivery: Principles and Applications, Amiji MM, ed. Boca Raton, FL: Taylor & Francis, 2005; pp. 4–30.

[25] Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm. Res. 2005; 22(1): 1–10.

[26] Nomura T, Koreeda N, Yamashita F, Takakura Y, Hashida M. Effect of particle size and charge on the disposition of lipid carriers after intratumoral injection into tissue-isolated tumors. Pharm. Res. 1998; 15(1): 128–132.

[27] Liang GF, Zhu YL, Sun B, Hu FH, Tian T, Li SC, et al. PLGA-based gene delivering nanoparticle enhance suppression effect of miRNA in HePG2 cells. Nanoscale Res. Lett. 2011; 6: 447.

[28] Jin S, Ye K. Mediated drug delivery and gene therapy. Biotechnol. Prog. 2007; 23: 32–41.

[29] Nitta SK, Numata K. Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. Int. J. Mol. Sci. 2013; 14: 1629-1654.

[30] Zhang X, Balazs DA, Godbey WT. Nanobiomaterials for nonviral gene delivery. In: Nanobiomaterials Handbook, Sitharaman B, ed. Taylor & Francis: CRC press, 2011; pp. 13–1 to 13–25.

[31] Dizaj SM, Jafari S, Khosroushahi AY. A sight on the current nanoparticle-based gene delivery vectors. Nanoscale Res. Lett. 2014, 9: 252.

[32] Adiseshaiah PP, Hall JB, McNeil SE. Nanomaterial standards for efficacy and toxicity assessment. Wiley Interdiscip. Rev. 2010; 2: 99–112.

[33] Gaumet M, Vargas A, Gurny R, Delie F. Nanoparticles for drug delivery: The need for precision in reporting particle size parameters. Eur. J. Pharm. Biopharm. 2008; 69: 1–9.
[34] Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J. Control. Release 2000; 65: 271–284.

[35] Mizrahy S, Peer D. Polysaccharides as building blocks for nanothereapeutics. Chem. Soc. Rev. 2012; 41: 2623–2640.

[36] Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. Nat. Rev. Drug Discov. 2010; 9: 615–627.

[37] Mohanraj V, Chen Y. Nanoparticles-A Review. Tropical J. Pharm. Res. 2006; 5: 561–573.

[38] Hedley ML, Curley J, Urban R. Microspheres containing plasmid-encoded antigens elicit cytotoxic T-cell responses. Nat. Med. 1998; 4: 365–368.

[39] Hermanson G. Bioconjugate Techniques. Academic Press Inc., San Diego, CA, 1996; pp. 570–592.

[40] Lu Y. Transcriptionally regulated, prostate-targeted gene therapy for prostate cancer. Adv. Drug Deliv. Rev. 2009; 61(7–8): 572–588.

[41] Conwell CC, Huang L. Recent advances in nonviral gene delivery. Adv. Genet. 2005; 53: 1–18.

[42] Niidome T, Huang L. Gene therapy progress and prospects: nonviral vectors. Gene Ther. 2002; 9(24):1647–1652.

[43] Bigger BW, Tolmachov O, Collombet JM, Fragkos M, Palaszewski I, Coutelle C. An araC-controlled bacterial cre expression system to produce DNA minicircle vectors for nuclear and mitochondrial gene therapy. J Biol. Chem. 2001; 276(25):23018–23027.

[44] Mayrhofer P, Schleef M, Jechlinger W. Use of minicircle plasmids for gene therapy. Methods Mol. Biol. 2009, 542: 87–104.

[45] Lee JM, Yoon TJ, Cho YS. Recent developments in nanoparticle-based siRNA delivery for cancer therapy. Biomed Res. Int. 2013; 782041.

[46] Nitta SK, Numata K. Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. Int. J. Mol. Sci. 2013; 14(1):1629–1654.

[47] Lee JM, Yoon TJ, Cho YS. Recent developments in nanoparticle-based siRNA delivery for cancer therapy. Biomed Res Int. 2013; 2013:782041. Doi: 10.1155/2013/782041.

[48] Athar M, Das AJ. Therapeutic nanoparticle: State of the art of Nanomedicine. Adv. Mater. Rev. 2014; 1(1): 25–37.

[49] Lubbe AS, Alexiou C, Bergemann C. Clinical applications of magnetic drug targeting. J Surg. Res. 2001; 95: 200–206.
[50] Scherer F, Anton M, Schillinger U, Henke J, Bergemann C, Krüger A, et al. Magneto-
fec tion: enhancing and targeting gene delivery by magnetic force in vitro and in vivo. Gene Ther. 2002; 9: 102–109.

[51] Plank C, Zelphati O, Mykhaylyk O. Magnetically enhanced nucleic acid delivery. Ten years of magnetofection - Progress and prospects. Adv. Drug. Deliv. Rev. 2011; 63(14–15): 1300–1303.

[52] Scherer F, Plank C. Magnetofection: Using magnetic particles and magnetic force to enhance and to target nucleic acid delivery. In: Smyth Templeton N, ed. Gene and Cell Therapy: Therapeutic Mechanisms and Strategies; 3rd Ed. Boca Raton, FL: CRC Press 2009; pp. 379–404.

[53] McBain SC, Yiu HHP, Dobson J. Magnetic nanoparticles for gene and drug delivery. Int. J. Nanomed. 2008; 3(2): 169–180.

[54] Buerli T, Pellegrino C, Baer K, Lardi-Studler B, Chudotvorova I, Fritschy JM, et al. Efficient transfection of DNA or shRNA vectors into neurons using magnetofection. Nat. Protoc. 2007; 2: 3090–101.

[55] Xenariou S, Griesenbach U, Ferrari S, Dean P, Scheule RK, Cheng SH, et al. Using magnetic forces to enhance non-viral gene transfer to airway epithelium in vivo. Gene Ther. 2006; 13: 1545–52.

[56] Fernandez-Pacheco R, Valdivia JG, Ibarra MR. Magnetic nanoparticles for local drug delivery using magnetic implants. Meth. Mol. Biol. 2009; 544: 559–69.

[57] McBain SC, Yiu HHP, Dobson J. Magnetic nanoparticles for gene and drug delivery. Int. J. Nanomed. 2008; 3(2): 169–180.

[58] Xu ZP, Zeng QH, Lu GQ, Yu AB. Inorganic nanoparticles as carriers for efficient cellular delivery. Chem. Eng. Sci. 2006; 61(3):1027–1040.

[59] Arruebo M, Pacheco RF, Ibarra MR, Santamaria J. Magnetic nanoparticle for drug delivery. Nano Today. 2007; 2(3): 22–32.

[60] Krotz F, de Wit C, Sohn HY, Zahler S, Gloe T, Pohl U, et al. Magnetofection:a highly efficient tool for antisense oligonucleotide delivery in vitro and in vivo. Mol. Ther. 2003; 7(5 Pt 1): 700–710.

[61] Krotz F, Sohn HY, Gloe T, Plank C, Pohl U. Magnetofection potentiates gene delivery to cultured endothelial cells. J. Vasc. Res. 2003; 40(5): 425–434.

[62] Kamau SW, Hassa PO, Hottiger MO. Enhancement of the efficiency of non-viral gene delivery by application of pulsed magnetic field. Nucleic Acids Res. 2006; 34(5): e40.

[63] Tiwari PK, Lee YS. Gene delivery in conjunction with gold nanoparticle and tumor treating electric field. J. Appl. Phys. 2013; 114(5): 054902.

[64] Alanazi AK, Radwan AA, Alsarra IA. Biopharmaceutical applications of nanogold. Saudi Pharm. J. 2010; 18: 179–193.
[65] Di Guglielmo C, Lopez DR, De Lapuente J, Mallafre JM, Suarez MB. Embryotoxicity of cobalt ferrite and gold nanoparticles: a first in vitro approach. Reprod. Toxicol. 2010; 30: 271–276.

[66] Kim D, Jon S. Gold nanoparticles in image-guided cancer therapy. Inorg. Chim. Acta. 2012; 393: 154–164.

[67] Stobiecka M, Hepel M. Double-shell gold nanoparticle-based DNA-carriers with poly-L-lysine binding surface. Biomaterials. 2011; 32: 3312–3321.

[68] Chithrani DB, Dunne M, Stewart J, Allen C, Jaffray DA. Cellular uptake and transport of gold nanoparticles incorporated in a liposomal carrier. Nanomed. Nanotechnol. Biol. Med. 2010; 6: 161–169.

[69] Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold nanoparticles for biology and medicine. Angewandte Chem. 2010; 49: 3280–3294.

[70] Papasani MR, Wang G, Hill RA. Gold nanoparticles: the importance of physiological principles to devise strategies for targeted drug delivery. Nanomed. Nanotechnol. Biol. Med. 2012; 8: 804–814.

[71] Ghosh R, Singh LC, Shohet JM, Gunaratne PH. A gold nanoparticle platform for the delivery of functional microRNAs into cancer cells. Biomaterials. 2013; 34: 807–816.

[72] Pissuwan D, Niidome T, Cortie MB. The forthcoming applications of gold nanoparticles in drug and gene delivery systems. J Con. Rel. 2011; 149: 65–71.

[73] Khan MS, Vishakante GD, Siddaramaiah H. Gold nanoparticles: a paradigm shift in biomedical applications. Adv. Colloid Interface Sci. 2013; 199–200: 44–58.

[74] Tarnawski R, Ulbricht M. Amphiphilic gold nanoparticles: Synthesis, characterization and adsorption to PEGylated polymer surfaces. Colloid Surface A: Physicochem. Engineer Aspects. 2011; 374: 13–21.

[75] Benkovicova M, Vegso K, Siffalovic P, Jergel M, Luby S, Majkova E. Preparation of gold nanoparticles for plasmonic applications. Thin Solid Films. 2013; 543:138–141.

[76] Khan AK, Rashid R, Murtaza G, Zahra A. Gold Nanoparticles: synthesis and applications in drug delivery. Trop. J Pharm. Res. 2014; 13(7): 1169–1177.

[77] Probst CE, Zrazhevskiy P, Bagalkot V, Gao X. Quantum dots as a platform for nanoparticle drug delivery vehicle design. Adv. Drug Deliv. Rev. 2013; 65(5): 703–718.

[78] Derfus AM, Chen AA, Bhatia SN, et al. Targeted quantum dot conjugates for siRNA delivery. Bioconjug. Chem. 2007; 18(5): 1391–1396.

[79] Qi L, Gao X. Emerging application of quantum dots for drug delivery and therapy. Expert Opin. Drug Deliv. 2008; 5(3): 263–267.

[80] Gulia S, Kakkar R. ZnO quantum dots for biomedical applications. Adv. Mater. Lett. 2013; 4(12): 876–887.
[81] Chen AA, Derfus AM, Khetani SA, Bhatia SN. Quantum dots to monitor RNAi delivery and improve gene silencing. Nucleic Acids Res. 2005; 33(22): e190.

[82] Dabbousi BO, Viejo RJ, Mikulec FV, Heine JR, Mattoussi H, Ober R, et al., (CdSe)ZnS core-shell quantum dots: synthesis and characterization of a size series of highly luminescent nanocrystallites. J. Phys. Chem. B. 1997; 101: 9463–9475.

[83] Modani S, Kharwade M, Nijhawan M. Quantum dots: a novelty of medical field with multiple applications. Int. J. Curr. Pharm. Res. 2013; 5(4): 55–59.

[84] Dey NS, Rao MEB. Quantum Dot: novel carrier for drug delivery. Int. J Res. Pharm. Biomed. Sci. 2011; 2(2): 448–458.

[85] Rathore KS, Lowalekar R, Nema RK. Quantum dots: a future drug delivery system. Pharma Rev. 2006; 4: 30–32.

[86] Ghasemi Y, Peymanib P, Afifi S. Quantum dot: magic nanoparticle for imaging, detection and targeting. Acta Biomed. 2009; 80: 156–165.

[87] Patel HR. Quantum dots: a novel technique for drug delivery and therapy 2007; available at http://www.pharmainfo.net/reviews/quantum-dots-novel-technique-drug-delivery-and-therapy. Accessed on 10 September 2014.

[88] Pandurangan DK, Mounika KS. Quantum dot aptamers: an emerging technology with wide scope in pharmacy. Int. J Pharm. Pharm. Sci. 2012; 4(3):24–31.

[89] Genger UR, Grabolle M, Jaricot SC, Nitschke R, Nann T. Quantum dots versus organic dyes as fluorescent labels. Nat. Methods 2008; 5(9):763–775.

[90] Mishra S, Tripathy P, Sinha SP. Advancements in the field of quantum dots. Int. J. Adv/Res. Tech. 2012; 1(3): 1–5.

[91] Jamiesona T, Bakhshi R, Petrovaa D, Pococka R, Imanib M, Seifaliana AM. Biological applications of quantum dots. Biomaterials. 2007; 28: 4717–4732.

[92] Drbohlavova J, Adam V, Kizek R, Hubalek J. Quantum dots: characterization, preparation and usage in biological systems. Int J. Mol. Sci. 2009;10:656–673.

[93] http://www.americanelements.com/quantum-dots.html

[94] Zrazhevskiyn P, Gao X. Multifunctional quantum dots for personalized medicine. Nano Today 2009; 4(5): 414–428.

[95] Wang Y, Chen L, Quantum dots, lighting up the research and development of nanomedicine. Nanomed: Nanotech. Biol. Med. 2011; 7: 385–402.

[96] Vengala P, Dasari A, Yeruva N, Quantum dots for drug delivery and therapy international. J Pharm. Tech. 2012; 4(2): 2055–2074.

[97] Mukherjee S, Das U, Quantum dots: an optimistic approach to novel therapeutics. Int. J. Pharm. Sci. Rev. Res. 2011; 7: 59–64.
[98] Cheki M, Moslehi M, Assadi M. Marvelous applications of quantum dots. Eur. Rev. Med. Pharmacol. Sci. 2013; 17: 1141–1148.

[99] Ozkan M. Quantum dots and other nanoparticles: what can they offer to drug discovery? Drug Discov. Today. 2004; 9: 1065–1071.

[100] Dorfs D, Krahne R, Falqui A, Manna L, Giannini C, Zanchet D. Quantum Dots: Synthesis and Characterization. In: Comprehensive Nanoscience and Technology, David LA, Gregory DS, Gary PW, eds. Amsterdam: Academic Press; pp. 219–270.

[101] Mokari T, Banin U. Synthesis and properties of CdSe/ZnS core/shell nanorods. Chem. Mater. 2003; 15: 3955–3960.
