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Polymer nanoparticles for targeted gene delivery

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Outline:

Introduction

Gene therapy is a medical intervention that uses genes for the treatment or prevention of disease. If the gene of interest is delivered properly to the desired site, then this strategy would allow the direct insertion of a gene into a specific cell. Gene therapy has gained massive researchers' interest because of its potential to be an alternative for surgery and drug treatments. Gene therapy have been applied to replace a mutated gene that causes disease, knocking out mutated genes, and introducing new genes into cells to help fight a disease.

The first attempts of gene therapy were focusing on the treatment of genetic disorders. In1989, tumor-infiltrating lymphocytes gene transfer was the first application of gene therapy on human. Moreover, patients with SCID (Severe Combined Immunodeficiency Defect) have been treated by gene therapy on the ADA gene in 1990.More recently, gene therapy is used to treat other diseases, such as autosomal dominant disorders, autosomal or X-linked recessive single gene disorders, polygenic disorders, specific cancer diseases, vascular disease, neurodegenerative disorders, and inflammatory conditions. Many methods of gene therapy have been used to treat numerous disorders[1,2].

Nanotechnology is one of the key technologies of the 21st century that merges material science and biotechnology; it is currently attracting the attention of many scientists all over the world. This field involves the utilization of biological systems such as cells, cellular components, and proteins, to manufacture efficient nanostructures. Nanotechnology is the new utensil that explores biomolecular structures, functions and properties. Bionanotechnology made it possible to determine structural elements of cells, molecular recognition and drug delivery [3–26].

Moreover, nanoparticles have been manipulated to perform as specific targets for therapies, as nano-vehicles to deliver certain therapeutic agents (Drugs, genetic material or a combination of both). Additionally, scientists have developed different types of nanoparticles, like carbon nanotubes, silicon oxides, metal oxides, nanocrystals, lipids, polymers, dendrimers, and quantum dots, together with increasing diversity of newly developed materials. These nanomaterials are modified and conjugated to biomolecules, so that they become highly biocompatible and specific targets to certain tissue. In addition, nanoparticles have an improved blood half-life and physiologic behaviour with insignificant side effects, and minimal or no toxicity to healthy tissues in living organisms.The optimal goal of nano drug delivery systems is to develop clinically useful tools for treating diseases in the clinic[3,15,27–59].

The field of nanotechnology in gene therapy is very promising and will revolutionize the therapeutics field especially for the treatment of genetic disorders and some types of cancer. It is an advanced translational research area facilitating translation of basic discoveries to the patients. The pharmaceutical industry is now giving a great deal of attention to commercialize new drug delivery systems especially for gene therapy. However, the process of clinical trials and Food and Drug Authorities is time consuming especially when new materials or chemicals are included in the new formulation. Hence, scientists are focusing on the improvement of existing dosage forms through the use of biocompatible biodegradable nanoparicles[57,60–68].

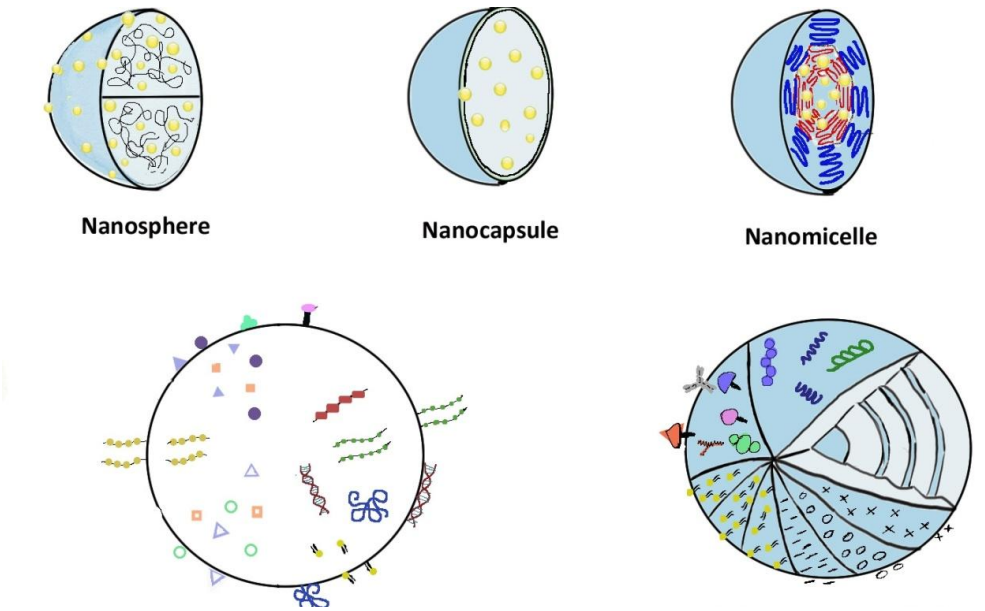

FIGURE 1.1
Schematic illustration of nanoparticles used for gene therapy

In this chapter we will shed the light on different aspects of gene therapy. We will discuss current and conventional methods of gene therapy. We will also elaborate on the advantages and disadvantages of the most commonly used methods of gene therapy. Additionally, polymer nanoparticles as gene therapy non-viral vectors will be discussed thoroughly, and the recent work in the field of polymer nanoparticles in gene therapy will be reported in this chapter. At the end of this chapter we will give some final remarks and recommendations on the optimal methods of gene delivery.

Gene therapy

Gene therapy is a medical intervention that uses genes for the treatment or prevention of disease. If the gene of interest is delivered properly to the desired site, then this strategy would allow the direct insertion of a gene into a specific cell. Gene therapy has gained massive researchers' interest because of its potential to be an alternative for surgery and drug treatments. Gene therapy has been applied to replace a mutated gene that causes disease, knocking out mutated genes, and introducing new genes into cells to help fight a disease.
In the same vein, gene therapy corrects cellular dysfunction and genetic mutations by delivering genomic materials into specific cells, gene delivery programs functional proteins by modifying the endogenous gene expression to produce a therapeutic effect. The use of messenger ribonucleic acid (mRNA) is widely used in gene transfer based therapies; in such cases a bulky piece of mRNA includes the promoter sequences that activate expression of the gene, the coding Sequences that direct production of a protein, and signaling sequences that direct RNA processing.

Alternatively, another method of gene therapy includes the down regulation/up regulation of a specific cellular gene. This can be achieved by transferring a relatively short piece of genetic material that is complementary to the mRNA. Gene expression can be affected through many blockage translational mechanisms, mRNA processing, or leading to destruction of the mRNA. The initial research interests in the field of gene therapy were on inherited genetic disorders. The first application of gene transfer in human was in tumor-infiltrating lymphocytes, and on immune deficient patients (SCID, Severe Combined Immunodeficiency Defect).

Gene Delivery methods and techniques have evolved heavily over the past few years, which resulted in many promising treatments for a vast number of disorders. There two main types of gene therapy; the Germline gene therapy and the Somatic gene therapy. The germline gene therapy corrects genetic abnormalities by direct manipulation of germline cells without specific targeting, however, this method has its own limitations. The direct germline cells manipulation has not been tested on human subjects for ethical restrictions. Furthermore, the somatic cell modifications have been applied on human subjects and showed promising outcomes. In the somatic gene therapy, genes are introduced to the diploid cells of the patient, where the genetic material is not relocated to its progeny. This kind of treatment can be classified into In Vitro delivery, In situ delivery and In vivo delivery[69–89].

Different gene delivery methods may be used in gene therapy to restore a specific gene function or silencing a special gene. The main aim of gene therapy is to develop a therapy of an appropriate material to repair a mutated gene. Even though gene therapy could be a promising treatment option for a number of diseases, its safety is still negotiable. Therefore, different types of biocompatible vectors have been used to deliver genes intended for gene therapy to overcome the disadvantages encountered with the traditional methods used for genetic material delivery.

Conventional methods of gene delivery

In gene therapy, the genetic material is transferred either through viral or non-viral delivery systems. The most commonly used viral vectors are derivatives from retrovirus, adenovirus, and adeno-associated virus (AAV). When considering gene delivery, three important criteria should be considered. First, the target site (tissue or cells) and its properties and its ability to be transduced. The second issue that should be considered in viral gene delivery, is the permanency of expression required, and lastly the size of the genetic material to be used in gene therapy should also be taken into consideration. In the next section, some of the most commonly used viral vectors will be described briefly.

Viral vectors used in gene therapy

Most of the currently available gene therapy is delivered via viral vectors. The viral vectors used in gene therapy are genetically modified to stop their reproduction which will lead to an enhanced safety. Even though the safety of viral vectors has been improved, they still exhibit many undesirable effects. For instance, viral vectors can induce immunological reactions, prompting the inflammatory system to produce toxins which might lead to mortality. Moreover, viral vectors are used for targeted delivery due to the specific receptors they possess, making it possible for the transfer of transgenes to other particular cells[90–106].

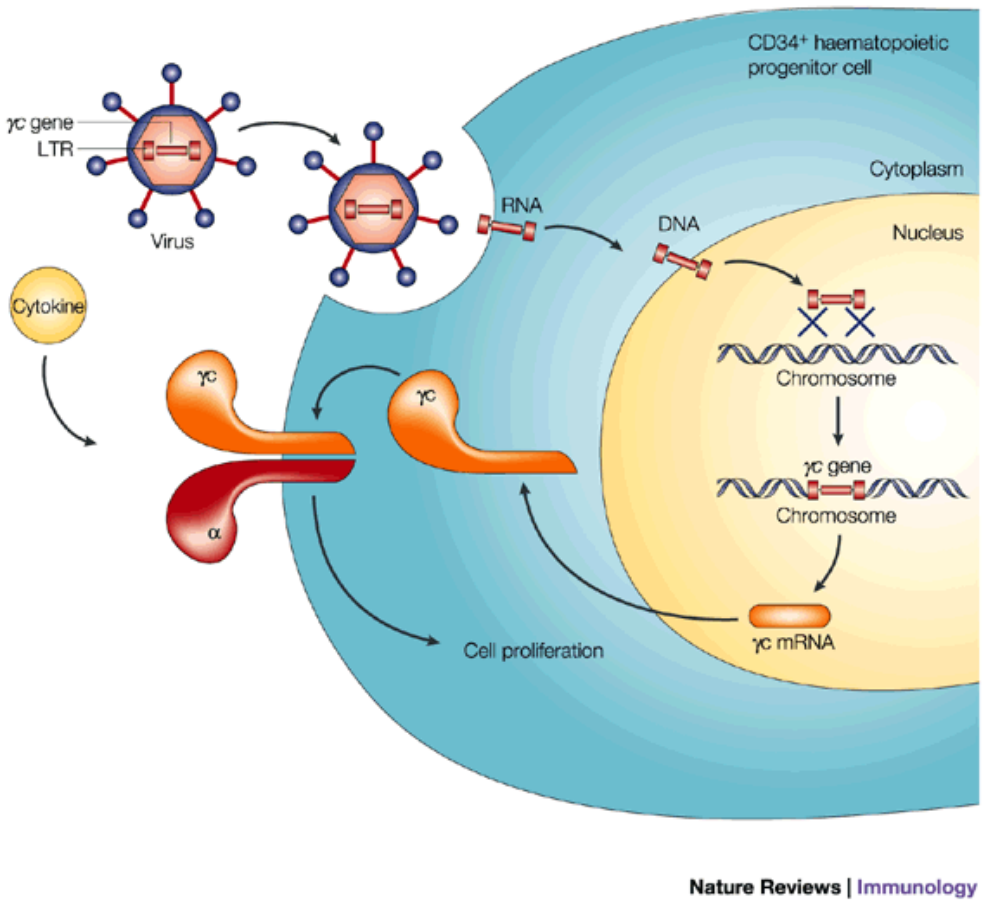

FIGURE 1.2
Schematic illustration of viral gene delivery. The virus binds to the cell, then its followed by cellular internalization of the virus. The viral RNA is retrotranscribed into DNA, to form a preintegration complex, then it recombines within the cell's genome[107]

Adeno Virus

Adenoviral vectors are extracted from a vast variety of species; more than 100 different serotypes have been identified. Most humans have been exposed to the adenovirus serotypes 2 and 5, which are mostly used as gene vectors. Furthermore, adenoviruses type 2 and 5 have low specificity to tissues and cells, hence it can transfer genes to a wider range of tissue types. In addition, adenoviruses have the capability to deliver large DNA particles. On the other hand, the use of adenoviruses in gene therapy is limited due to the immunological responses induced in many tissue. Adenoviruses have caused serious side effects in patients, and in some cases have caused death[95,95,108–110].

Adeno associated viral vectors

Adeno-associated vectors (AAV) are considered safer than adenoviral vectors, because of the lack of pathogenicity and replication. In human, AAVs are able to integrate into a specific site on chromosome 19 with no noticeable expression in vivo. AAVs have been successfully used in the treatment of some diseases, such as CF, hemophilia B, Leber congenital amaurosis, and AAT (Alpha-1 antitrypsine) deficiency. The main drawback of this type of gene delivery is their restricted transgene capacity (up to 4.8 kb)[111,112].

Helper-dependent adenoviral vector

The Helper-dependent adenoviral vector (HdAd), consists of two vectors; the helper, which contains all the viral genes required for replication but has genetic defect in the packaging domain. The other vector comprises the ends of the viral genome, therapeutic gene sequences, and the normal packaging recognition signal. The HdAd vector is an optimised version of the adenovirus. Therefore, many of the disadvantages encountered with the first-generation adenovirus has been overcome. the packaging capacity has been improved, no immunogenicity, and reduced toxicity. A more developed form of the adenovirus is the Hybrid adenoviral vectors; The Hybrid adenoviral vectors are a hybrid between adenovirus and retrovirus that shows improved features and high stability[112–115].

Retroviral vectors

Retroviral vectors have an advantage over types of viral vectors, they have the ability to pass through the nuclear pores of mitotic cells, hence it is capable to transfect dividing cells making them prime candidates for in situ and ex vivo treatments. Retrovirus is the most common viral vectors used for gene delivery especially in germline and somatic gene therapy[90,97,98,104,105].

Lentiviruses

Lentiviruses have the ability to integrate with non-dividing cells which gives them unique features over retroviruses. Lentiviruses are a subclass of retroviruses, they have the capacity to deliver 8 kb of sequence. They have high-efficiency infection of dividing and nondividing cells, they also have high stability expression of a transgene, low immunogenicity, and the capacity to transfer larger transgenes. Plus, lentiviruses are extensively used for *ex vivo* gene transfer in central nervous system with no significant undesired effects. Lentiviruses have been applied in the treatment of neurological disorders, like, Alzheimer, Huntington's disease, lysosomal storage diseases, and spinal injury[70,99,100,102,103].

Non Viral gene delivery methods

The nonviralgene delivery include cationic liposomes and polymers, or physical methods, such as gene gun, electroporation, particle bombardment, ultrasound utilization, and magnetofection.

Naked DNA

This technique although widely investigated, its efficiency is low compared with other methods of

gene delivery. And it is only suitable for specific applications. Naked DNA transfer is limited to some cells like cardiac muscles, skeletal muscle, skin where small genes are injected directly into the cells[116,117].

DNA particle bombardant by gene gun

This method has been developed to replace the naked DNA delivery. In this technique, gold micro beads are attached with plasmid DNA and then targeted through a gas pressure gun where the genetic material penetrates into the target tissue cells[118–127].

Electroporation

In this method the DNA is incorporated into the cells through an electrical current. Electroporation can be applied in vivo on different types of tissue, and it has been used in cancer treatment. The major drawback of technique is the requirement of surgery to insert the electrodes into internal tissue, and the damage that may be caused by the high voltage, as it may harmorgans[128–133].

Other non- viral gene delivery methods

The hydrodynamic gene transfer has shown promising results in vivo, it is an efficient and uncomplicated process for intracellular delivery of genetic material[134–138]. Another non-viral gene delivery method, is the Ultrasound. Ultrasound facilitates the internalization of DNA particles by making nanopores in the membrane, the applications of this method is limited due to its low efficiency[139]. Magnetofection, is a gene delivery technique where a magnetic field is applied to concentrate Iron Oxide particles containing nucleic acid desired target. In this way, the magnetic force allows a rapid concentration of the entire applied vector dose onto cells[140–145].

Polymer nanoparticles in gene therapy

A major limitation of gene therapy is the exposure of genetic material to nucleases, which hinders this kind of therapy to achieve its desired therapeutic effect. Using the conventional methods of direct gene delivery or vector based delivery, a number of obstacles stands in the way of localizing the nucleic acids into the cell nucleus. Even though gene therapy could be a promising treatment option for a number of diseases, its safety is still negotiable. Therefore, different types of biocompatible nanoparticles have been used to deliver genes intended for gene therapy to overcome the disadvantages encountered with the traditional methods used for genetic material delivery.

In fact, viral vectors exhibit major safety issues like antigenicity, off site targeting, and inflammation. An optimal gene delivery system is one that guarantees the delivery of the genetic material to the target site with high specificity and high efficiency; with minimal side effects. Nanoparticles (NPs) are nonviral gene delivery systems. Their unique nano structure provides them with properties that allows the incorporation of genetic materials and drugs. Plus, the surface of the NPs could be modified with different functional groups to allow efficient penetration and specific targeting[3,17,50–53,146–157].

Polymer nanoparticles (PNPs) deliver genes or therapeutic proteins including drugs which can either be dissolved or encapsulated within them forming a nanoparticle and a nanocapsule

respectively. PNPs can also deliver proteins to the targeted cells by entrapping them within its structure forming a nanosphere. The delivered therapeutic proteins or drugs act by altering defective proteins or genes in the patient's cells. The size of the polymer nanoparticle could be tuned to enable these drugs and therapeutic protein to fit in. PNPs, like all nanoparticles are capable of regaining their size once inside the cell through the physiological change in pH[13,57,60–63,157–167].

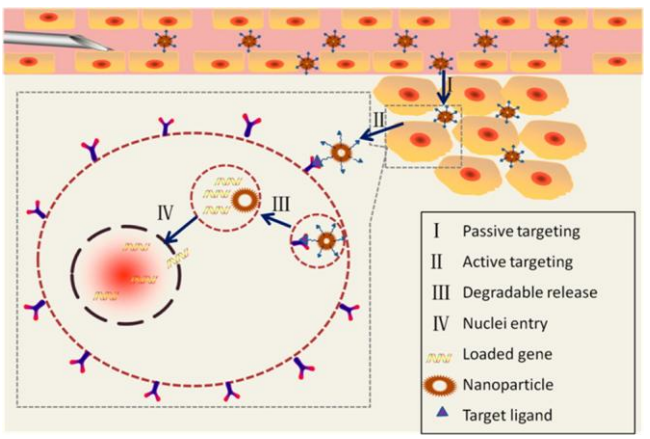

FIGURE 1.3
Mechanism of the delivery of genes using NPs[58]

PNPs have been utilized in drug delivery, where they have shown high biocompatibility and high encapsulation capacity. They are great candidates for gene delivery, because they are highly stable and they offer controlled release of active ingredients. Also, PNPs can be used for targeted delivery by surface modification, and they allow the delivery of combined active materials. PNPs are synthesized from non-toxic biodegradable, biocompatible polymers like, Chitosan, cyclodextrin, polyethyleneimine (PEI), poly(lactic-co-glycolic) acid (PLGA), and dendrimers.

PNPs have facilitated the development of new treatment methods with improved efficacy for treating diseases which had once been viewed as incurable like genetic, immunological and neural disorders. In some cases, the delivered genes act by enhancing the functions of the cells. Polymer nanoparticles are used to overcome the various challenges that have been encountered in using gene therapy. Some genes have relatively long base sequences which make it difficult for them to be delivered to the desired sites. To fit into the target cell, the DNA must be condensed into the nanostructures, to permit their internalization within the cells. In some cases, gene silencing may also arise as the target cells may act against the delivered genes[14,58,59,64–66,169–182].

Putnam et al, have demonstrated that using polycations such as polylysine can overcome the DNA size barrier as it "can condense DNA into toroidal nanostructures" to sizes less than 150 nm which can internalized within the cell. Researchers have also identified various ways in overcoming the challenge of separation of the DNA from the carrier. Using nanoparticles to conjugate the DNA, researchers have developed an effective way to ensure that the genes are delivered to the targeted cells[81].

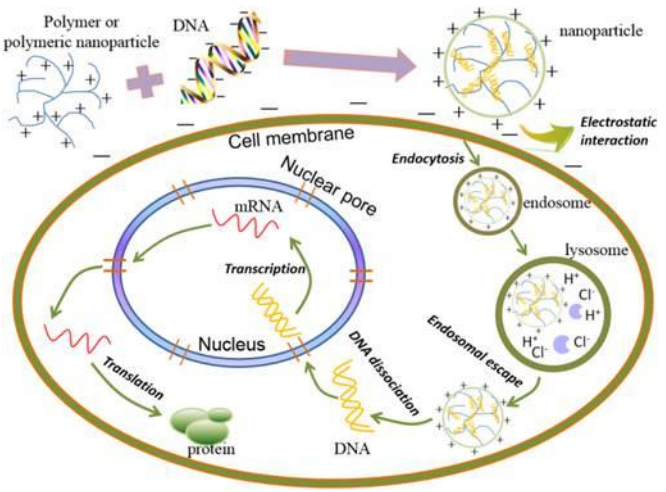

FIGURE 1.4
Schematic illustration of the internalization of PEI polymer nanoparticles loaded with DNA[168]

Mohammedi et al. have synthesized DNA-Chitosan nanoparticles to deliver DNA to the Lung Epithelial cells[174]. Also, in 2014Tang et al. have utilized chitosan based (PNPs) Trimethylatedchitosan has been synthesized as gene delivery systems, TMC-g-PCL/DNA polyplexeshave shown high uptake efficiency than PEI/DNA polyplexes[3183]. Plus, Das et al have utilized PEI based nanoparticles to deliver siRNA to STAT3 in lung cancer, in vitro and in vivo [51]. Other research groups have also synthesized chitosan as the main targeting nanoparticles for siRNA delivery to treat different diseases like, lung cancer, ovarian cancer, pancreatic cancer and hepatocellular carcinoma[3,53,57,63,159,177,180,184]. In 2015, Bishop et al. have utilized polymer coated gold nanoparticles for DNA and siRNA delivery, where this type of inorganic nanoparticles have shown good results in gene silencing[154]. Colombo et al have synthesized hybrid lipid-polymer nanoparticles for siRNA delivering[55]. While, other up to date studies have shown the improved cancer treatments obtained with co delivery[52,150,156,158,162,164,175].

Conclusions

Many gene delivery methods have seen the light over the past three decades. The gene delivery systems are either viral or nonviral delivery systems. These gene delivery methods exhibit side effects and have their own limitations, hence, some of the methods mentioned in this chapter have not yet had clinical applications. Yet, some of the gene delivery systems have showed great potential when studied in vitro and in vivo and show promising results to be further investigated on specific cells and tissues. Non viral delivery systems are still in a juvenile stage of research, more *in vivo* studies are required in this field. Major improvements on the currently available systems; refining the extracellular targeting and delivery, improving the intracellular delivery, and minimizing toxicity and side effects on human body.

Polymer nanoparticles have led to an enhanced development of gene therapy different diseases in the past years. The rise of many biocompatible materials led to the development of gene therapy systems that will revolutionize the field of gene therapy. NPs can be great alternative of the conventional viral and nonviral gene delivery methods. The gene targeting using biocompatible NPs

will definitely result in an enhanced patient treatment of various diseases and disorders. Moreover, the use of polymer nanoparticles in gene delivery have shown to have less undesirable effects and better targeting. The synthesis methods of PNPs, the polymers used, and surface functionalization should all be taken into account to get the therapeutic effectiveness of a therapeutic NP.

Clinical evaluations are extremely significant and are not yet widely investigated. The current outcomes are inadequate to make a final opinion regarding the effectiveness of NP based gene therapy. Therefore, toxicity studies in vitro and in vivo are needed so that researchers can translate this advanced basic research to the bedside. In addition, toxicological studies "Nanotoxicology" has focused on the safety of nanoparticles based therapies, however, only few studies have been reported so far.

In conclusion, the realization of PNPs gene therapy still needs further proof of concept. Moving from the lab to the clinic has not yet been achieved. In the future, research in this area still requires in depth studies that involve functional assays. The nanomaterial should be designed and characterized; then, the routes of administration of the PNPs gene therapies should be confirmed and finally, the synthesis methods should be streamlined in order for the formulations to be replicated at the industrial level.

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References

1. Tandon, A. *et al.* BMP7 Gene Transfer via Gold Nanoparticles into Stroma Inhibits Corneal Fibrosis In Vivo. *PLoS ONE***8,** e66434 (2013).
2. Banwait, J. K. &Bastola, D. R. Contribution of bioinformatics prediction in microRNA-based cancer therapeutics. *MiRNAs Targets Cancer Treat. Ther. Des. Deliv.***81,** 94–103 (2015).
3. Tang, S. *et al.* Design and formulation of trimethylated chitosan-graft-poly(ε-caprolactone) nanoparticles used for gene delivery. *Carbohydr. Polym.***101,** 104–112 (2014).
4. Liu, J. *et al.* pH-Sensitive nano-systems for drug delivery in cancer therapy. *Biotechnol. Nanomedicine***32,** 693–710 (2014).
5. Conde, J. *et al.* Gold-nanobeacons for gene therapy: evaluation of genotoxicity, cell toxicity and proteome profiling analysis. *Nanotoxicology***8,** 521–532 (2014).
6. Wang, F., Willner, B. &Willner, I. DNA nanotechnology with one-dimensional self-assembled nanostructures. *Curr. Opin. Biotechnol.***24,** 562–574 (2013).
7. Lin, E.-H. *et al.*Polyethyleneimine and DNA nanoparticles-based gene therapy for acute lung injury. *Nanomedicine Nanotechnol. Biol. Med.***9,** 1293–1303 (2013).
8. Vimal, S. *et al.* Chitosan tripolyphosphate (CS/TPP) nanoparticles: Preparation, characterization and application for gene delivery in shrimp. *Acta Trop.***128,** 486–493 (2013).

9. Ahmed, M. &Douek, M. The Role of Magnetic Nanoparticles in the Localization and Treatment of Breast Cancer. *BioMed Res. Int.***2013,** (2013).
10. Zheng, S. W. *et al.* Preparation and characterization of magnetic gene vectors for targeting gene delivery. *Appl. Surf. Sci.***259,** 201–207 (2012).
11. Yoshizawa, S. Micro and nanotechnological tools for study of RNA. *Biochimie***94,** 1588–1594 (2012).
12. Xu, J., Ganesh, S. &Amiji, M. Non-condensing polymeric nanoparticles for targeted gene and siRNA delivery. *Int. J. Pharm.***427,** 21–34 (2012).
13. Shmueli, R. B., Sunshine, J. C., Xu, Z., Duh, E. J. & Green, J. J. Gene delivery nanoparticles specific for human microvasculature and macrovasculature. *Nanomedicine Nanotechnol. Biol. Med.***8,** 1200–1207 (2012).
14. Shim, M. S. & Kwon, Y. J. Stimuli-responsive polymers and nanomaterials for gene delivery and imaging applications. *Adv. Drug Deliv. Rev.***64,** 1046–1059 (2012).
15. Shan, Y. *et al.* Gene delivery using dendrimer-entrapped gold nanoparticles as nonviral vectors. *Biomaterials***33,** 3025–3035 (2012).
16. Mannell, H. *et al.* Site directed vascular gene delivery in vivo by ultrasonic destruction of magnetic nanoparticle coated microbubbles. *Nanomedicine Nanotechnol. Biol. Med.***8,** 1309–1318 (2012).
17. Liang, Y. *et al.* Delivery of cationic polymer-siRNA nanoparticles for gene therapies in neural regeneration. *Biochem. Biophys. Res. Commun.***421,** 690–695 (2012).
18. Haque, F. *et al.*Ultrastable synergistic tetravalent RNA nanoparticles for targeting to cancers. *Nano Today***7,** 245–257 (2012).
19. Sun, T.-M. *et al.* Cancer stem cell therapy using doxorubicin conjugated to gold nanoparticles via hydrazone bonds. *Biomaterials***35,** 836–845 (2014).
20. Li, C. *et al.* In vivo real-time visualization of tissue blood flow and angiogenesis using Ag2S quantum dots in the NIR-II window. *Biomaterials***35,** 393–400 (2014).
21. Cywinski, P. J., Moro, A. J. &Löhmannsröben, H.-G. Cyclic GMP recognition using ratiometric QD-fluorophore conjugate nanosensors. *Biosens. Bioelectron.***52,** 288–292 (2014).
22. Ai, J., Xu, Y., Lou, B., Li, D. & Wang, E. Multifunctional AS1411-functionalized fluorescent gold nanoparticles for targeted cancer cell imaging and efficient photodynamic therapy. *Talanta***118,** 54–60 (2014).
23. Wang, J. *et al.*Photostable water-dispersible NIR-emitting CdTe/CdS/ZnS core–shell–shell quantum dots for high-resolution tumor targeting. *Biomaterials***34,** 9509–9518 (2013).
24. Tang, Y. *et al.* The role of surface chemistry in determining in vivo biodistribution and toxicity of CdSe/ZnS core–shell quantum dots. *Biomaterials***34,** 8741–8755 (2013).
25. Lu, Y. *et al.* In vivo behavior of near infrared-emitting quantum dots. *Biomaterials***34,** 4302–4308 (2013).
26. Ding, Y. *et al.* The performance of thiol-terminated PEG-paclitaxel-conjugated gold nanoparticles. *Biomaterials***34,** 10217–10227 (2013).
27. Noriega-Luna, B., Godinez, L., Rodriguez, F. & Rodriguez, A. Applications of Dendrimers in Drug Delivery Agents, Diagnosis, Therapy, and Detection.
28. Kesharwani, P., Jain, K. & Jain, N. K. Dendrimer as nanocarrier for drug delivery. *Top. Issue BiorelatedPolym.***39,** 268–307 (2014).
29. Baker, J. R. Dendrimer-based nanoparticles for cancer therapy. *ASH Educ. Program Book***2009,** 708–719 (2009).

30. Marvaniya, H. M., Parikh, P., Patel, V., Modi, K. &Jjyoti Sen, D. Dendrimer Nanocarriers as Versatile Vectors in Gene Delivery. *J. Chem. Pharm. Res.***2,** 97–108 (2010).
31. Castillo, G. *et al.* Detection of aflatoxin B1 by aptamer-based biosensor using PAMAM dendrimers as immobilization platform. *Food Control***52,** 9–18 (2015).
32. Tang, Y. *et al.* Efficient in Vitro siRNA Delivery and Intramuscular Gene Silencing Using PEG-Modified PAMAM Dendrimers. *Mol. Pharm.***9,** 1812–1821 (2012).
33. Shah, V. *et al.* Genotoxicity of Different Nanocarriers: Possible Modifications for the Delivery of Nucleic Acids. *Curr. Drug Discov. Technol.***10,** 8–15 (2013).
34. Dahlman, J. E. *et al.* In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. *Nat Nano***9,** 648–655 (2014).
35. Liao, H.-W. &Yau, K.-W. In vivo gene delivery in the retina using polyethylenimine. *BioTechniques***42,** 285–288 (2007).
36. Twibanire, J. K. &Grindley, T. B. Polyester Dendrimers: Smart Carriers for Drug Delivery. *Polyester***6,** 179–213 (2014).
37. Wolinsky, J. B. &Grinstaff, M. W. Therapeutic and diagnostic applications of dendrimers for cancer treatment. *Des. Dev. Strateg. Polym. Mater. Drug Gene Deliv. Appl.***60,** 1037–1055 (2008).
38. Sun, Y., Jiao, Y., Wang, Y., Lu, D. & Yang, W. The strategy to improve gene transfection efficiency and biocompatibility of hyperbranched PAMAM with the cooperation of PEGylated hyperbranched PAMAM. *Int. J. Pharm.***465,** 112–119 (2014).
39. Ghosh, R., Singh, L. C., Shohet, J. M. &Gunaratne, P. H. A gold nanoparticle platform for the delivery of functional microRNAs into cancer cells. *Biomaterials***34,** 807–816 (2013).
40. Lee, S. H., Bae, K. H., Kim, S. H., Lee, K. R. & Park, T. G. Amine-functionalized gold nanoparticles as non-cytotoxic and efficient intracellular siRNA delivery carriers. *Int. J. Pharm.***364,** 94–101 (2008).
41. Bishop, C. J., Tzeng, S. Y. & Green, J. J. Degradable polymer-coated gold nanoparticles for co-delivery of DNA and siRNA. *ActaBiomater.***11,** 393–403 (2015).
42. Kim, J.-H. *et al.* Effective delivery of anti-miRNA DNA oligonucleotides by functionalized gold nanoparticles. *J. Biotechnol.***155,** 287–292 (2011).
43. Noh, S. M. *et al.* Enhanced cellular delivery and transfection efficiency of plasmid DNA using positively charged biocompatible colloidal gold nanoparticles. *Biochim. Biophys. Acta BBA - Gen. Subj.***1770,** 747–752 (2007).
44. Kim, D.-W. *et al.* Modulation of biological processes in the nucleus by delivery of DNA oligonucleotides conjugated with gold nanoparticles. *Biomaterials***32,** 2593–2604 (2011).
45. Dizaj, S. M., Jafari, S. &Khosroushahi, A. Y. A sight on the current nanoparticle-based gene delivery vectors. *Nanoscale Res. Lett.***9,** 252–252 (2014).
46. Lin, Q., Chen, J., Zhang, Z. & Zheng, G. Lipid-based Nanoparticles in the Systemic Delivery of siRNA. *Nanomed.***9,** 105–120 (2014).
47. Buyens, K. *et al.* Liposome based systems for systemic siRNA delivery: Stability in blood sets the requirements for optimal carrier design. *J. Controlled Release***158,** 362–370 (2012).
48. Sonoke, S. *et al.* Tumor Regression in Mice by Delivery of Bcl-2 Small Interfering RNA with Pegylated Cationic Liposomes. *Cancer Res.***68,** 8843–8851 (2008).
49. Tomalia, D. A., Naylor, A. M. & Goddard, W. A. Starburst Dendrimers: Molecular-Level Control of Size, Shape, Surface Chemistry, Topology, and Flexibility from Atoms to Macroscopic Matter. *Angew. Chem. Int. Ed. Engl.***29,** 138–175 (1990).

50. Yang, F. *et al.* Anti-tumor effects in mice induced by survivin-targeted siRNA delivered through polysaccharide nanoparticles. *Biomaterials***34,** 5689–5699 (2013).
51. Das, J. *et al.* Assessment of drug delivery and anticancer potentials of nanoparticles-loaded siRNA targeting STAT3 in lung cancer, in vitro and in vivo. *Toxicol. Lett.***225,** 454–466 (2014).
52. Zhao, X. *et al.* Co-delivery of HIF1α siRNA and gemcitabine via biocompatible lipid-polymer hybrid nanoparticles for effective treatment of pancreatic cancer. *Biomaterials***46,** 13–25 (2015).
53. Ragelle, H. *et al.* Chitosan nanoparticles for siRNA delivery: Optimizing formulation to increase stability and efficiency. *J. Controlled Release***176,** 54–63 (2014).
54. Ruponen, M., Ylä-Herttuala, S. &Urtti, A. Interactions of polymeric and liposomal gene delivery systems with extracellular glycosaminoglycans: physicochemical and transfection studies. *Biochim. Biophys. Acta BBA - Biomembr.***1415,** 331–341 (1999).
55. Colombo, S. *et al.* Mechanistic profiling of the siRNA delivery dynamics of lipid–polymer hybrid nanoparticles. *J. Controlled Release***201,** 22–31 (2015).
56. Pittella, F. *et al.* Pancreatic cancer therapy by systemic administration of VEGF siRNA contained in calcium phosphate/charge-conversional polymer hybrid nanoparticles. *J. Controlled Release***161,** 868–874 (2012).
57. Xie, Y. *et al.* PEGylated carboxymethyl chitosan/calcium phosphate hybrid anionic nanoparticles mediated hTERT siRNA delivery for anticancer therapy. *Biomaterials***35,** 7978–7991 (2014).
58. Lin, G., Zhang, H. & Huang, L. Smart Polymeric Nanoparticles for Cancer Gene Delivery. *Mol. Pharm.***12,** 314–321 (2015).
59. Gavrilov, K. & Saltzman, W. M. Therapeutic siRNA: principles, challenges, and strategies. *Yale J. Biol. Med.***85,** (2012).
60. Yokoyama, M. Gene delivery using temperature-responsive polymeric carriers. *Drug Discov. Today***7,** 426–432 (2002).
61. Shi, J. *et al.* Hybrid lipid–polymer nanoparticles for sustained siRNA delivery and gene silencing. *Nanomedicine Nanotechnol. Biol. Med.***10,** 897–900 (2014).
62. He, C., Yin, L., Tang, C. & Yin, C. Multifunctional polymeric nanoparticles for oral delivery of TNF-α siRNA to macrophages. *Biomaterials***34,** 2843–2854 (2013).
63. Han, L., Tang, C. & Yin, C. Oral delivery of shRNA and siRNA via multifunctional polymeric nanoparticles for synergistic cancer therapy. *Biomaterials***35,** 4589–4600 (2014).
64. Kirtane, A. R. &Panyam, J. Polymer nanoparticles: Weighing up gene delivery. *Nat Nano***8,** 805–806 (2013).
65. Zhang, Y. *et al.* The development of an in vitro assay to screen lipid based nanoparticles for siRNA delivery. *J. Controlled Release***174,** 7–14 (2014).
66. Desai, P. R. *et al.* Topical delivery of anti-TNFα siRNA and capsaicin via novel lipid-polymer hybrid nanoparticles efficiently inhibits skin inflammation in vivo. *J. Controlled Release***170,** 51–63 (2013).
67. Chen, Y., Gao, D.-Y. & Huang, L. In vivo delivery of miRNAs for cancer therapy: Challenges and strategies. *MiRNAs Targets Cancer Treat. Ther. Des. Deliv.***81,** 128–141 (2015).
68. Chen, Y., Gao, D.-Y. & Huang, L. In vivo delivery of miRNAs for cancer therapy: Challenges and strategies. *MiRNAs Targets Cancer Treat. Ther. Des. Deliv.***81,** 128–141 (2015).
69. Wolf, E. *et al.* Transgenic technology in farm animals--progress and perspectives. *Exp. Physiol.***85,** 615–625 (2000).

70. Abbasi, H. *et al.* Lentiviral vector-mediated transduction of goat undifferentiated spermatogonia. *Anim. Reprod. Sci.***163,** 10–17 (2015).
71. Kumar Pramod, R., Kumar, R. &Mitra, A. Transgenic expression of green fluorescent protein in caprine embryos produced through electroporation-aided sperm-mediated gene transfer. *Gene***576,** 505–511 (2016).
72. Moreira, P. *et al.* Transgenic mouse offspring generated by ROSI. *J. Reprod. Dev.* (2015). doi:10.1262/jrd.2015-105
73. Gun, G. &Kues, W. A. Current progress of genetically engineered pig models for biomedical research. *BioResearch Open Access***3,** 255–264 (2014).
74. Barkalina, N. *et al.* Effects of mesoporous silica nanoparticles upon the function of mammalian sperm in vitro. *Nanomedicine Nanotechnol. Biol. Med.***10,** 859–870 (2014).
75. Chandrashekran, A. *et al.* Efficient generation of transgenic mice by lentivirus-mediated modification of spermatozoa. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.***28,** 569–576 (2014).
76. Yuan, Y. *et al.* Generation of fertile offspring from Kit(w)/Kit(wv) mice through differentiation of gene corrected nuclear transfer embryonic stem cells. *Cell Res.***25,** 851–863 (2015).
77. Moreira, P. N. &Montoliu, L. Intracytoplasmic sperm injection (ICSI)-mediated transgenesis in mice. *Methods Mol. Biol. Clifton NJ***1194,** 141–156 (2014).
78. Liu, Y. *et al.* In vitro manipulation techniques of porcine embryos: a meta-analysis related to transfers, pregnancies and piglets. *Reprod. Fertil. Dev.* (2014). doi:10.1071/RD13329
79. Chiou, P. P. *et al.* Production of homozygous transgenic rainbow trout with enhanced disease resistance. *Mar. Biotechnol. N. Y. N***16,** 299–308 (2014).
80. Xin, N. *et al.* The impact of exogenous DNA on the structure of sperm of olive flounder (Paralichthysolivaceus). *Anim. Reprod. Sci.***149,** 305–310 (2014).
81. Nagatomo, H. *et al.* Comparing spatial expression dynamics of bovine blastocyst under three different procedures: in-vivo, in-vitro derived, and somatic cell nuclear transfer embryos. *Jpn. J. Vet. Res.***63,** 159–171 (2015).
82. Romagnolo, D. F., Papoutsis, A. J., Laukaitis, C. &Selmin, O. I. Constitutive expression of AhR and BRCA-1 promoter CpGhypermethylation as biomarkers of ERalpha-negative breast tumorigenesis. *BMC Cancer***15,** 1026 (2015).
83. Hosseini, S. M. *et al.* Epigenetic modification with trichostatin A does not correct specific errors of somatic cell nuclear transfer at the transcriptomic level; highlighting the non-random nature of oocyte-mediated reprogramming errors. *BMC Genomics***17,** 16 (2016).
84. Marcotte, R. *et al.* Functional Genomic Landscape of Human Breast Cancer Drivers, Vulnerabilities, and Resistance. *Cell***164,** 293–309 (2016).
85. Singh, V. K., Kumar, N., Kalsan, M., Saini, A. & Chandra, R. Mechanism of Induction: Induced Pluripotent Stem Cells (iPSCs). *J. Stem Cells***10,** 43–62 (2015).
86. Freedman, B. S. Modeling Kidney Disease with iPS Cells. *Biomark. Insights***10,** 153–169 (2015).
87. Yang, Y. *et al.* Naive Induced Pluripotent Stem Cells Generated From beta-Thalassemia Fibroblasts Allow Efficient Gene Correction With CRISPR/Cas9. *Stem Cells Transl. Med.***5,** 8–19 (2016).
88. Halabi, N. M. *et al.* Preferential Allele Expression Analysis Identifies Shared Germline and Somatic Driver Genes in Advanced Ovarian Cancer. *PLoS Genet.***12,** e1005755 (2016).

89. Toledo, R. A. *et al.* Recurrent mutations of chromatin remodeling genes and kinase receptors in pheochromocytomas and paragangliomas. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* (2015). doi:10.1158/1078-0432.CCR-15-1841
90. Wu, H. L. *et al.* Identification and spontaneous immune targeting of an endogenous retrovirus K envelope protein in the Indian rhesus macaque model of human disease. *Retrovirology***13,** 6 (2016).
91. Chen, R., Kobewka, M., Addison, W., Lachance, G. & Tyrrell, D. L. Intrinsic Viral Factors Are the Dominant Determinants of the Hepatitis C Virus Response to Interferon Alpha Treatment in Chimeric Mice. *PloS One***11,** e0147007 (2016).
92. Ho, B.-C., Yang, P.-C. & Yu, S.-L. MicroRNA and Pathogenesis of Enterovirus Infection. *Viruses***8,** (2016).
93. Katz, M. G. *et al.* Mitigation of myocardial fibrosis by molecular cardiac surgery-mediated gene overexpression. *J. Thorac. Cardiovasc. Surg.* (2015). doi:10.1016/j.jtcvs.2015.11.031
94. Ziehr, B., Lenarcic, E., Cecil, C. & Moorman, N. J. The eIF4AIII RNA helicase is a critical determinant of human cytomegalovirus replication. *Virology***489,** 194–201 (2016).
95. Brown, N. J. & Hirsch, M. L. Adeno-associated virus (AAV) gene delivery in stem cell therapy. *Discov. Med.***20,** 333–342 (2015).
96. Jang, S. H., Lee, S. & Chung, H. Y. Characterization of Leukemia-Inducing Genes Using a Proto-Oncogene/HomeoboxGene Retroviral Human cDNA Library in a Mouse In Vivo Model. *PloS One***10,** e0143240 (2015).
97. Erlwein, O. *et al.* Determination of sequences required for HERV-K transduction and its recognition by foreign retroviral virions. *J. Virol.* (2015). doi:10.1128/JVI.02731-15
98. Suerth, J. D. *et al.* Efficient generation of gene-modified human natural killer cells via alpharetroviral vectors. *J. Mol. Med. Berl. Ger.***94,** 83–93 (2016).
99. Zhou, Q. *et al.* Exclusive Transduction of Human CD4+ T Cells upon Systemic Delivery of. *J. Immunol. Baltim. Md 1950***195,** 2493–2501 (2015).
100. Hoban, M. D., Orkin, S. H. & Bauer, D. E. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. *Blood* (2016). doi:10.1182/blood-2015-09-618587
101. Qi, Z. &Mi, R. Inhibition of human telomerase reverse transcriptase in vivo and in vitro for retroviral vector-based antisense oligonucleotide therapy in ovarian cancer. *Cancer Gene Ther.***23,** 36–42 (2016).
102. Pala, F. *et al.* Lentiviral-mediated gene therapy restores B cell tolerance in Wiskott-Aldrich syndrome patients. *J. Clin. Invest.***125,** 3941–3951 (2015).
103. Houghton, B. C., Booth, C. & Thrasher, A. J. Lentivirus technologies for modulation of the immune system. *Curr. Opin. Pharmacol.***24,** 119–127 (2015).
104. Demeulemeester, J., De Rijck, J., Gijsbers, R. &Debyser, Z. Retroviral integration: Site matters: Mechanisms and consequences of retroviral integration site selection. *BioEssays News Rev. Mol. Cell. Dev. Biol.***37,** 1202–1214 (2015).
105. Twitty, C. G. *et al.* Retroviral Replicating Vectors Deliver Cytosine Deaminase Leading to Targeted. *Hum. Gene Ther. Methods* (2015). doi:10.1089/hgtb.2015.106
106. Serrao, E. &Engelman, A. N. Sites of retroviral DNA integration: From basic research to clinical applications. *Crit. Rev. Biochem. Mol. Biol.* 1–17 (2015). doi:10.3109/10409238.2015.1102859
107. Fischer, A., Hacein-Bey, S. &Cavazzana-Calvo, M. Gene therapy of severe combined immunodeficiencies. *Nat Rev Immunol***2,** 615–621 (2002).

108. Aoyama, Y., Kobayashi, K., Morishita, Y., Maeda, K. &Murohara, T. Wnt11 gene therapy with adeno-associated virus 9 improves the survival of mice with myocarditis induced by coxsackievirus B3 through the suppression of the inflammatory reaction. *J. Mol. Cell. Cardiol.***84,** 45–51 (2015).
109. Freytag, S. O. *et al.* Prospective Randomized Phase 2 Trial of Intensity Modulated Radiation Therapy With or Without Oncolytic Adenovirus-Mediated Cytotoxic Gene Therapy in Intermediate-Risk Prostate Cancer. *Int. J. Radiat. Oncol.***89,** 268–276 (2014).
110. Aoyama, Y., Kobayashi, K., Morishita, Y., Maeda, K. &Murohara, T. Wnt11 gene therapy with adeno-associated virus 9 improves the survival of mice with myocarditis induced by coxsackievirus B3 through the suppression of the inflammatory reaction. *J. Mol. Cell. Cardiol.***84,** 45–51 (2015).
111. Hirsch, M. L., Wolf, S. J. &Samulski, R. J. Delivering Transgenic DNA Exceeding the Carrying Capacity of AAV Vectors. *Methods Mol. Biol. Clifton NJ***1382,** 21–39 (2016).
112. Vidovic, D. *et al.* Noninvasive Imaging Reveals Stable Transgene Expression in Mouse Airways After Delivery of a Nonintegrating Recombinant Adeno-Associated Viral Vector. *Hum. Gene Ther.* (2015). doi:10.1089/hum.2015.109
113. van der Loo, J. C. M. & Wright, J. F. Progress and challenges in viral vector manufacturing. *Hum. Mol. Genet.* (2015). doi:10.1093/hmg/ddv451
114. Aboshiha, J. *et al.* Preserved outer retina in AIPL1 Leber's congenital amaurosis: implications for gene therapy. *Ophthalmology***122,** 862–864 (2015).
115. Ashtari, M. *et al.* Plasticity of the human visual system after retinal gene therapy in patients with Leber's congenital amaurosis. *Sci. Transl. Med.***7,** 296ra110 (2015).
116. Remaut, K. *et al.* Aerosolized Non-viral Nucleic Acid Delivery in the Vaginal Tract of Pigs. *Pharm. Res.***33,** 384–394 (2016).
117. Stoller, F. *et al.* Hepatocyte Transfection in Small Pigs After Weaning by Hydrodynamic Intraportal Injection of Naked DNA/Minicircle Vectors. *Hum. Gene Ther. Methods***26,** 181–192 (2015).
118. Lin, C.-C. *et al.* Delivery of noncarrier naked DNA vaccine into the skin by supersonic flow induces a polarized T helper type 1 immune response to cancer. *J. Gene Med.***10,** 679–689 (2008).
119. Lowe, B. A. *et al.* Enhanced single copy integration events in corn via particle bombardment using low quantities of DNA. *Transgenic Res.***18,** 831–840 (2009).
120. Cao, Y. *et al.* Gene gun bombardment with DNA-coated golden particles enhanced the protective effect of a DNA vaccine based on thioredoxin glutathione reductase of Schistosoma japonicum. *BioMed Res. Int.***2013,** 952416 (2013).
121. Chang, M.-L. *et al.* Gene gun bombardment with DNA-coated gold particles is a potential alternative to hydrodynamics-based transfection for delivering genes into superficial hepatocytes. *Hum. Gene Ther.***19,** 391–395 (2008).
122. Indurker, S., Misra, H. S. &Eapen, S. Genetic transformation of chickpea (Cicer arietinum L.) with insecticidal crystal protein gene using particle gun bombardment. *Plant Cell Rep.***26,** 755–763 (2007).
123. Sparks, C. A. & Jones, H. D. Genetic transformation of wheat via particle bombardment. *Methods Mol. Biol. Clifton NJ***1099,** 201–218 (2014).
124. Shiva Prakash, N. *et al.* Marker-free transgenic corn plant production through co-bombardment. *Plant Cell Rep.***28,** 1655–1668 (2009).
125. Liu, G., Campbell, B. C. & Godwin, I. D. Sorghum genetic transformation by particle bombardment. *Methods Mol. Biol. Clifton NJ***1099,** 219–234 (2014).

126. Sailaja, M., Tarakeswari, M. & Sujatha, M. Stable genetic transformation of castor (Ricinuscommunis L.) via particle gun-mediated gene transfer using embryo axes from mature seeds. *Plant Cell Rep.***27,** 1509–1519 (2008).
127. Uchida, M., Li, X. W., Mertens, P. &Alpar, H. O. Transfection by particle bombardment: delivery of plasmid DNA into mammalian cells using gene gun. *Biochim. Biophys. Acta***1790,** 754–764 (2009).
128. Lambricht, L. *et al.* Clinical potential of electroporation for gene therapy and DNA vaccine delivery. *Expert Opin. Drug Deliv.* (2015). doi:10.1517/17425247.2016.1121990
129. Vergara, M. N., Gutierrez, C. & Canto-Soler, M. V. Efficient Gene Transfer in Chick Retinas for Primary Cell Culture Studies: An Ex-ovo Electroporation Approach. *J. Vis. Exp. JoVE* (2015). doi:10.3791/52002
130. SharifiTabar, M. *et al.* Evaluating Electroporation and Lipofectamine Approaches for Transient and Stable Transgene Expressions in Human Fibroblasts and Embryonic Stem Cells. *Cell J.***17,** 438–450 (2015).
131. Gibot, L. &Rols, M.-P. Gene transfer by pulsed electric field is highly promising in cutaneous wound healing. *Expert Opin. Biol. Ther.* 1–11 (2015). doi:10.1517/14712598.2016.1098615
132. Ding, X.-F. & Fan, M. Nonviral Gene Therapy of the Nervous System: Electroporation. *Methods Mol. Biol. Clifton NJ***1382,** 297–305 (2016).
133. Kumar Pramod, R., Kumar, R. &Mitra, A. Transgenic expression of green fluorescent protein in caprine embryos produced through electroporation-aided sperm-mediated gene transfer. *Gene***576,** 505–511 (2016).
134. McNeill, E. *et al.* Hydrodynamic Gene Delivery of CC Chemokine Binding Fc Fusion Proteins to Target Acute Vascular Inflammation In Vivo. *Sci. Rep.***5,** 17404 (2015).
135. Kamimura, K. *et al.* Image-Guided Hydrodynamic Gene Delivery: Current Status and Future Directions. *Pharmaceutics***7,** 213–223 (2015).
136. Pedersen, L. *et al.* Restoration of Haemoglobin Level Using Hydrodynamic Gene Therapy with Erythropoietin Does Not Alleviate the Disease Progression in an Anaemic Mouse Model for TGFbeta1-Induced Chronic Kidney Disease. *PloS One***10,** e0128367 (2015).
137. Wu, Y. *et al.* The dynamic impact of hydrodynamic gene transfer on the immune system. *Int. J. Clin. Exp. Med.***8,** 8540–8550 (2015).
138. Ju, H.-L., Han, K.-H., Lee, J. D. & Ro, S. W. Transgenic mouse models generated by hydrodynamic transfection for genetic studies of liver cancer and preclinical testing of anti-cancer therapy. *Int. J. Cancer J. Int. Cancer* (2015). doi:10.1002/ijc.29703
139. Mead, B. P. *et al.* Targeted gene transfer to the brain via the delivery of brain-penetrating DNA nanoparticles with focused ultrasound. *J. Control. Release Off. J. Control. Release Soc.***223,** 109–117 (2015).
140. Prosen, L. *et al.*Magnetofection: a reproducible method for gene delivery to melanoma cells. *BioMed Res. Int.***2013,** 209452 (2013).
141. Wang, Y. *et al.* A magnetic nanoparticle-based multiple-gene delivery system for transfection of porcine kidney cells. *PloS One***9,** e102886 (2014).
142. Sukoyan, M. A. *et al.*Magnetofection of human somatic cells with magnetite and cobalt ferrospinel nanoparticles. *Bull. Exp. Biol. Med.***154,** 673–676 (2013).
143. Al-Deen, F. N., Selomulya, C., Ma, C. &Coppel, R. L. Superparamagnetic nanoparticle delivery of DNA vaccine. *Methods Mol. Biol. Clifton NJ***1143,** 181–194 (2014).

144. Pickard, M. R., Adams, C. F., Barraud, P. & Chari, D. M. Using magnetic nanoparticles for gene transfer to neural stem cells: stem cell propagation method influences outcomes. *J. Funct. Biomater.***6,** 259–276 (2015).
145. Nayerossadat, N., Maedeh, T. & Ali, P. A. Viral and nonviral delivery systems for gene delivery. *Adv. Biomed. Res.***1,** 27 (2012).
146. Birmingham, A. *et al.* 3[prime] UTR seed matches, but not overall identity, are associated with RNAi off-targets. *Nat Meth***3,** 199–204 (2006).
147. Navarro, G. &Tros de ILarduya, C. Activated and non-activated PAMAM dendrimers for gene delivery in vitro and in vivo. *Nanomedicine Nanotechnol. Biol. Med.***5,** 287–297 (2009).
148. Hamilton, A. J. &Baulcombe, D. C. A Species of Small Antisense RNA in Posttranscriptional Gene Silencing in Plants. *Science***286,** 950–952 (1999).
149. Jinek, M. &Doudna, J. A. A three-dimensional view of the molecular machinery of RNA interference. *Nature***457,** 405–412 (2009).
150. Yhee, J. Y. *et al.* Cancer-targeted MDR-1 siRNA delivery using self-cross-linked glycol chitosan nanoparticles to overcome drug resistance. *J. Controlled Release***198,** 1–9 (2015).
151. Pan, X. *et al.* Cationic lipid-coated magnetic nanoparticles associated with transferrin for gene delivery. *Int. J. Pharm.***358,** 263–270 (2008).
152. Kwok, A. & Hart, S. L. Comparative structural and functional studies of nanoparticle formulations for DNA and siRNA delivery. *Nanomedicine Nanotechnol. Biol. Med.***7,** 210–219 (2011).
153. Gao, L.-Y. *et al.* Core-Shell type lipid/rPAA-Chol polymer hybrid nanoparticles for in vivo siRNA delivery. *Biomaterials***35,** 2066–2078 (2014).
154. Bishop, C. J., Tzeng, S. Y. & Green, J. J. Degradable polymer-coated gold nanoparticles for co-delivery of DNA and siRNA. *ActaBiomater.***11,** 393–403 (2015).
155. Liang, Y. *et al.* Delivery of cationic polymer-siRNA nanoparticles for gene therapies in neural regeneration. *Biochem. Biophys. Res. Commun.***421,** 690–695 (2012).
156. Zhong, J. *et al.* Development of hybrid-type modified chitosan derivative nanoparticles for the intracellular delivery of midkine-siRNA in hepatocellular carcinoma cells. *Hepatobiliary Pancreat. Dis. Int.***14,** 82–89 (2015).
157. Guo, P. *et al.* Engineering RNA for Targeted siRNA Delivery and Medical Application. *Adv. Drug Deliv. Rev.***62,** 650–666 (2010).
158. Han, S. *et al.* Effects of hydrophobic core components in amphiphilic PDMAEMA nanoparticles on siRNA delivery. *Biomaterials***48,** 45–55 (2015).
159. Li, T. S. C., Yawata, T. &Honke, K. Efficient siRNA delivery and tumor accumulation mediated by ionically cross-linked folic acid–poly(ethylene glycol)–chitosan oligosaccharide lactate nanoparticles: For the potential targeted ovarian cancer gene therapy. *Eur. J. Pharm. Sci.***52,** 48–61 (2014).
160. Jackson, A. L. *et al.* Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotech***21,** 635–637 (2003).
161. Reynolds, A. *et al.* Induction of the interferon response by siRNA is cell type- and duplex length-dependent. *RNA N. Y. N***12,** 988–993 (2006).
162. Tang, S. *et al.* Inhibition of metastasis and growth of breast cancer by pH-sensitive poly (β-amino ester) nanoparticles co-delivering two siRNA and paclitaxel. *Biomaterials***48,** 1–15 (2015).

163. Jeong, J. H., Kim, S. W. & Park, T. G. Molecular design of functional polymers for gene therapy. *Prog. Polym. Sci.***32,** 1239–1274 (2007).
164. Zheng, W. *et al.* Multifunctional polyamidoamine-modified selenium nanoparticles dual-delivering siRNA and cisplatin to A549/DDP cells for reversal multidrug resistance. *ActaBiomater.***11,** 368–380 (2015).
165. Wang, H.-X., Xiong, M.-H., Wang, Y.-C., Zhu, J. & Wang, J. N-acetylgalactosamine functionalized mixed micellar nanoparticles for targeted delivery of siRNA to liver. *J. Controlled Release***166,** 106–114 (2013).
166. Tzeng, S. Y. *et al.* Non-viral gene delivery nanoparticles based on Poly(β-amino esters) for treatment of glioblastoma. *Biomaterials***32,** 5402–5410 (2011).
167. Russ, V., Günther, M., Halama, A., Ogris, M. & Wagner, E. Oligoethylenimine-grafted polypropylenimine dendrimers as degradable and biocompatible synthetic vectors for gene delivery. *J. Controlled Release***132,** 131–140 (2008).
168. Jin, L., Zeng, X., Liu, M., Deng, Y. & He, N. Current progress in gene delivery technology based on chemical methods and nano-carriers. *Theranostics***4,** 240–255 (2014).
169. Yang, Y. *et al.* Poly(imidazole/DMAEA)phosphazene/DNA self-assembled nanoparticles for gene delivery: Synthesis and in vitro transfection. *J. Controlled Release***127,** 273–279 (2008).
170. Putnam, D., Gentry, C. A., Pack, D. W. & Langer, R. Polymer-based gene delivery with low cytotoxicity by a unique balance of side-chain termini. *Proc. Natl. Acad. Sci.***98,** 1200–1205 (2001).
171. Carlisle, R. C. *et al.* Polymer-coated polyethylenimine/DNA complexes designed for triggered activation by intracellular reduction. *J. Gene Med.***6,** 337–344 (2004).
172. Steinbach, J. M., Weller, C. E., Booth, C. J. & Saltzman, W. M. Polymer nanoparticles encapsulating siRNA for treatment of HSV-2 genital infection. *J. Controlled Release***162,** 102–110 (2012).
173. Qiang, B. *et al.*Poly(methylidene malonate 2.1.2) nanoparticles: a biocompatible polymer that enhances peri-adventitial adenoviral gene delivery. *J. Controlled Release***98,** 447–455 (2004).
174. Mohammadi, Z. *et al.* Preparation and evaluation of chitosan–DNA–FAP-B nanoparticles as a novel non-viral vector for gene delivery to the lung epithelial cells. *Int. J. Pharm.***409,** 307–313 (2011).
175. Han, J. *et al.* Preparation of novel curdlan nanoparticles for intracellular siRNA delivery. *Carbohydr. Polym.***117,** 324–330 (2015).
176. Behlke, M. A. Progress towards in vivo use of siRNAs. *Mol. Ther. J. Am. Soc. Gene Ther.***13,** 644–670 (2006).
177. Lee, J. Y. *et al.* Prolonged gene silencing by siRNA/chitosan-g-deoxycholic acid polyplexes loaded within biodegradable polymer nanoparticles. *J. Controlled Release***162,** 407–413 (2012).
178. Zamore, P. D., Tuschl, T., Sharp, P. A. &Bartel, D. P. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell***101,** (2000).
179. Hornung, V. *et al.* Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. *Nat. Med.***11,** 263–270 (2005).
180. Malhotra, M., Tomaro-Duchesneau, C. & Prakash, S. Synthesis of TAT peptide-tagged PEGylated chitosan nanoparticles for siRNA delivery targeting neurodegenerative diseases. *Biomaterials***34,** 1270–1280 (2013).

181. Lee, M. S. *et al.* Target-specific delivery of siRNA by stabilized calcium phosphate nanoparticles using dopa–hyaluronic acid conjugate. *J. Controlled Release***192,** 122–130 (2014).
182. Lee, S. H., Choi, S. H., Kim, S. H. & Park, T. G. Thermally sensitive cationic polymer nanocapsules for specific cytosolic delivery and efficient gene silencing of siRNA: Swelling induced physical disruption of endosome by cold shock. *J. Controlled Release***125,** 25–32 (2008).
183. Choi, K., Jang, M., Kim, J. H. &Ahn, H. J. Tumor-specific delivery of siRNA using supramolecular assembly of hyaluronic acid nanoparticles and 2b RNA-binding protein/siRNA complexes. *Biomaterials***35,** 7121–7132 (2014).
184. Vauthier, C., Zandanel, C. & Ramon, A. L. Chitosan-based nanoparticles for in vivo delivery of interfering agents including siRNA. *Curr. Opin. Colloid Interface Sci.***18,** 406–418 (2013).