Nanoparticles in Gene Therapy

Milad Soleimani, Aaesha Majid Al Zaabi, Maxime Merheb, Rachel Matar*

Biotechnology Department, American University of Ras Al Khaimah, Ras Al Khaimah, United Arab Emirates


Abstract

In less than two decades, gene therapy has evolved to become a significant technique to treat a wide variety of genetic disorders, and a promising approach to replace the use of chemical drugs and surgeries in a more effective way. Vectors used to deliver genes into target cells are many; each type of vectors is discussed briefly in this study, considering the methods, the advantages, and limitations of each type of vectors individually. A major approach discussed is the use of Nanoparticles as non-viral carriers for gene delivery purposes regarding their favorable characteristics such as their nano-metric size, high surface to volume ratio, stability, their ability of undergoing surface modifications, their ability to encapsulate nucleic acids and release them inside target cells, and their lack of immunogenicity. In this study, we are presenting a general review on nanoparticles use in gene therapy. The main types of nanoparticles are explained in details, including liposomes, chitosan-based nanoparticles, dendrimers, and gold nanoparticles. Each type of nanoparticles is discussed based on the structure, function, and the successful attempts using these types of particles in different gene therapy purposes and in several diseases treatments referring to some considerable studies and experiments done in vitro and in vivo. Toxic effects and potential hazards of nanoparticles are in addition to the ethical issues regarding the use of nanoparticles in gene therapy.

Keywords: Gene Therapy; Nanoparticles; Non-viral carriers.

INTRODUCTION

In recent years, Gene therapy has become a powerful therapeutic approach for many different diseases, including diabetes and cancer (Niu et al., 2008). Appropriately, gene therapy using genetic engineering, and gene-delivery systems have been broadly studied (Daamen et al., 2007). Among scientists, it is a major challenge to engineer effective gene-delivery vectors with less cytotoxicity (Gawande et al., 2015). Viral vectors, which have been used as gene-delivery carriers, have shown many signs of toxicity and side effects (Bouard et al., 2009). Therefore, non-viral vectors used for gene delivery have been studied and developed to overcome the physiological barriers of the viral vectors (Ramamoorth and Narvekar, 2015). Cationic lipids have an excellent gene-incorporation ability, high transfection efficiency, and ease of preparation; those properties make them ideal non-viral gene carriers.

Nevertheless, cationic lipids’ clinical applications have some limitations, such as the poor reproducibility caused by high batch-to-batch variations, and their instability (Zhi et al., 2010). Instead, biodegradable polymeric nanoparticles have been studied for many reasons; they have a small particle size with an excellent physiological stability, ease of surface functionalization, and sustained-release profiles. But, one significant limitation of the polymeric nanoparticles is that they have a low transfection efficiency, which constrains their clinical success (Sharma et al., 2015). This review will discuss various types of nanoparticles and their applications in gene therapy as well as the technical and ethical concerns surrounding their use in gene therapy.

Gene therapy and the methods of gene delivery

Gene therapy refers to a mode of treatment that involves introducing new genes into cells, repairing or replacing existing abnormal genes, or regulating the expression of particular genes (Matar et al., 2015). There are two types of gene therapy, namely somatic gene therapy and germline gene therapy. In brief, somatic gene therapy involves treating diseases by...
genetically modifying somatic cells such that the changes made are only limited to the patient (Wang and Gao, 2014). Germline gene therapy, on the other hand, aims to correct genetic disorders in either reproductive cells such as ova and sperm or early embryos, and therefore, any genetic alterations introduced via this type of gene therapy are inheritable (Nielsen, 1997). Generally, it is possible to accomplish gene therapy by introducing naked DNA into target cells; however, some nucleic acid-based medicines are not able to cross the cellular membrane by simple passive diffusion methods because of the negative charge of large DNA molecules and the negative nature of the cellular membrane. Therefore, it is important to use a vector to assist the progress of transferring DNA molecules into the cell. Both somatic gene therapy and germline gene therapy, whether in vitro or in vivo, require vectors in order to insert genes into cells. Vectors are broadly classified as viral and non-viral vectors (Lundstrom, 2003). Due to their natural ability to transduce their own genetic material into host cells, viral vectors have been shown to be a highly efficient delivery system in gene therapy (Bouard et al., 2009). Furthermore, viral vectors possess high packaging capacities of up to 50kb and various types of single- and double-stranded genetic material, which significantly widens their range of therapeutic applications (Mountain, 2000). Cancer (Trepel et al., 2015), neurological disorders (Choong et al., 2015), retinal diseases (Ye et al., 2015), hemophilia (High and Anguela, 2015), and arthritis (Goater et al., 2000) are only a small proportion of a vast array of diseases that can be treated using viral vectors. Some of the drawbacks of viral vectors, however, are the difficulty associated with their scale-up and engineering and their tendency to exhibit immunotoxicity (Touchefeu et al., 2010). Non-viral vectors constitute all forms of non-viral delivery systems, ranging from physical methods such as electroporation (Dean et al., 2003), ultrasound (Chen and Hwang, 2013), and magnetofection (Plank et al., 2003) to chemically built vehicles such as nanoparticles and polymers. As far as their advantages over viral vectors, non-viral vectors are safer in that they are less likely to cause immune reactions and mutagenesis and are also more cost-effective and easier to produce (Ramamooirth and Narvekar, 2015).

Nanoparticles

Nanotechnologists define a particle as a small object that moves and behaves as a single unit. Particles are conveniently classified as fine and ultrafine particles that range in size from 100 nm to 2500 nm and from 1 nm to 100 nm, respectively (Buzea et al., 2007). Nanoparticles belong to the latter class of particles with at least one dimension smaller than 1 μm. Some believe that they ought to be designated a new state other than the conventional liquid, solid, gaseous, and plasma states due to their exceptionally large surface area and quantum size effects (Buzea et al., 2007). Despite having a unique state, nanoparticles are restricted in form to being either (non-aggregating) amorphous or crystalline (Buzea et al., 2007). The methods by which nanomaterials are manufactured can be reduced to two principal modes of fabrication: top-down nanofabrication that involves disintegrating large (bulk) structures into smaller units, and bottom-up nanofabrication that involves constructing nanostructures with individual atoms (Helland, 2006).

Types of Nanoparticles

A number of different types of nanoparticles (Table 1) have been synthesized using methods derived from the above two fundamental modes of nanofabrication. Nanoparticles can be classified into organic and inorganic nanoparticles. Organic vectors include polymers and lipid complexes, whereas inorganic vectors include, but are not limited to, metal nanoparticles and semiconductor nanoparticles (Mohanraj et al., 2006).

Organic Nanoparticles

The two main types of organic nanoparticles are lipid-based nanoparticles and polymers. Lipid-based nanoparticles (lipoplexes) can be divided into cationic lipids, cationic liposomes, cationic solid lipids, and cationic emulsions. Cationic lipids are positive amphiphilic molecules with four main components, a cationic polar head group, a hydrophobic chain, a spacer, and a backbone. The role of the cationic polar head group is that it can form self-assembly interactions with DNA (Zhi et al., 2010). The physical properties (e.g., flexibility) of the lipid bilayer are affected by the hydrophobic chain, which accordingly impacts the gene transfer efficiency of the vector. There is a spacer between the cationic polar head group and the hydrophobic chain which influences the determination of gene transfection efficiency, the biodegradability, and the chemical stability. The last constituent of the cationic lipids is the backbone that is usually glycerol-based, and works as a scaffold (Koynova and Tenchov, 2010). Cationic liposomes are spherical multilayered vesicles composed of either natural or synthetic phospholipids and steroids or other surfactants. They are produced by agitating or sonicating lipid sheets whereupon small stretches separate and self-assemble to form liposomes. Owing to their ability to fuse with biological membranes, liposomes have been successfully employed to transfect DNA into cells and deliver antibiotics, neurotransmitters, etc. (Akbarzadeh et al., 2013). Core-shell nanoparticles are consisted of a core and a shell. The core is composed of lipids that have high melting points, and the shell is composed of surfactants. Those structures can be deemed promising vectors due to their inconsiderable risk of toxicity at high doses, and their low transformation efficiency (Gawande et al., 2015).
Nanoparticles in Gene Therapy

Solid lipid nanoparticles (SLNs) are lipid-based colloidal systems that have a solid matrix at the body temperature. The structure of SLNs is such that a solid hydrophobic core (usually composed of triglycerides) is surrounded by a phospholipid coating (Rawat et al., 2006). SLNs can be synthesized in a two-step process: by creating an oil-in-water emulsion and solidifying the lipid phase through dispersion. Similar to liposomes, SLNs find several applications such as ocular (Attama et al., 2007) and pulmonary (Liu et al., 2008) drug delivery and transfection of genetic material (Pragati et al., 2009). Cationic lipid emulsions are built using a cationic lipid covering a hydrophobic oil phase. Their nano-sized range, low toxicity, physical stability, biodegradability, and biocompatibility make them affirmative carriers to deliver genes to targeted cells. This was demonstrated by Yi et al. who achieved efficient transfection using serum-resistant cationic lipid emulsions formulated with soybean oil and 1,2-dioleoyl-sn-glycero-3-trimethylammonium-propane (DOTAP) as a cationic emulsifier (Yi et al., 2000).

Polymer-based nanoparticles (polyplexes) are neutralized, nano-based polymers that fall under the two classes of cationic polymers and biopolymers. Cationic polymers can interact with anionic genetic material owing to the primary, secondary, tertiary and/or quaternary amino groups in their backbone that can bind DNA electrostatically (Eliyahu et al., 2005). These polyplex/DNA complexes are internalized into cells via adsorptive endocytosis or receptor-mediated endocytosis and finally move into lysosomes (Li et al., 2012). To improve their transfection efficiency, one needs to modify their molecular weight, surface charge, charge density, and/or hydrophilicity (Sun et al., 2010). Biopolymers are polymers produced by living organisms. These biocompatible and non-immunogenic nanoparticles are fabricated from materials primarily made of proteins and polysaccharides (Nitta and

Table 1: Structure, properties, and successful attempts of different nanoparticles.

<table>
<thead>
<tr>
<th>Type of nanoparticles</th>
<th>Structure</th>
<th>Properties</th>
<th>Successful attempts/treatments</th>
</tr>
</thead>
</table>
| liposomes             | -Spherical multi-layered vesicles.  
                        -composed of natural or synthetic phospholipids and steroids. | -Ability to fuse with biological membranes. | -Used to deliver FUSI to lung cancer cells in vivo.  
                        -Result: decrease in tumor size. |
| Chitosan-based        | -Linear, cationic, binary, hetero-polysaccharide derived by alkaline de-N-acetylation of chitin. | -Has a polyelectrolyte nature which allows it to make strong electrostatic interactions with DNA and negatively charged mucosal surfaces.  
                        -Capabilities of forming stable complexes with DNA as small as 20-500 nm in diameter.  
                        -Its binding efficiency is related to its deacetylation degree and MW. | -Gastrointestinal administration of chitosan-insulin gene complexes to diabetic rats.  
                        -Result: dramatic decline in the level of fasting blood sugar of the rats injected with chitosan-DNA particles. |
| Dendrimers            | -Monodisperse structure.  
                        -Regularly-shaped highly branched 3D architecture.  
                        -composed of an initiator core, interior layers of polymers, and an exterior functionally layer attached to the outermost interior generations. | -High transfection efficiency (PAMAM Dendrimers).  
                        -Has amine groups which function in DNA binding and promoting DNA cellular uptake (primary amines), and reinforce the release of DNA into the cytoplasm by acting as a proton sponge in endosomes (tertiary amines). | -Used for long-term treatment of Parkinson’s disease when mixed with plasmid DNA carrying GDNF gene.  
                        -Result: An improvement in the number of line crossings and inactive time, and the loss of dopaminergic neurons was reduced. |
| Gold nanoparticles    | - Inertness.  
                        -Ease of functionalization with thiol linkages.  
                        - Plasmon resonance. | -Inhibiting the transcription of T7 RNA polymerase using gold nanoparticles functionalized with trimethylammonium thiol.  
                        -Using gold nanoparticle mediated (GNOME) laser transfection technique to improve the transfection efficiency of gold nanoparticles. |
Protein-based biopolymers are highly stable, nutritional, and biodegradable carriers with such unique abilities as gelation, emulsification, and water binding capacity (Elzoghby et al., 2012). Fibronectin (Kim et al., 2010), collagen (Wallace and Rosenblatt, 2003), and elastin (Daamen et al., 2007) are some of the most commonly used naturally derived protein-based biopolymers. Genetic engineering has offered an opportunity to further control the composition of protein-based biopolymers by encoding their sequences at the genetic level. A widely popular family of genetically engineering biopolymers is that of elastin-like polypeptides (ELP). They are composed of repeats of sequence Val-Pro-Gly-Xaa-Gly, where Xaa can be substituted with any amino acid except proline (Kowalczyk et al., 2014). What makes ELPs particularly stand out from their natural counterparts is their tendency to undergo inverse temperature phase transition in response to environmental changes (MacEwan and Chilkoti, 2010). This property mainly depends on the molecular weight and the amino acid sequence of ELPs. A technique known as recursive directional ligation (RDL), introduced by Meyer and Chilkoti, is usually used to build multiple libraries of ELPs with varying molecular weights (Meyer and Chilkoti, 2002). A convenient way to perform RDL is through plasmid reconstruction, whereby an oligonucleotide harboring a DNA monomer with two restriction sites is subjected to repeated rounds of enzymatic cleavage and ligation in an attempt to obtain repetitive sequences of the original DNA monomer (McDaniel et al., 2010). Similar to protein-based biopolymers, polysaccharide-based biopolymers also exhibit high degrees of biocompatibility and biodegradability, and are quite amenable to chemical modification (Mizrahy and Peer, 2012). Based on native charge, they can be divided into cationic, anionic, and nonionic biopolymers (Nitta and Numata, 2013). Chitosan is currently the most studied polysaccharide-based biopolymer. It is a linear, cationic, binary, hetero-polysaccharide derived by alkaline de-N-acetylation of chitin (Koping-Hoggard et al., 2001). Moreover, it possesses a polyelectrolyte nature that allows it to make strong electrostatic interactions with DNA and negatively charged mucosal surfaces. It has been demonstrated that chitosan is also capable of forming stable complexes with DNA as small as 20-500 nm in diameter. The binding efficiency of chitosan is related to its deacetylation degree and molecular weight (Mansouri et al., 2004). Recently, a hybrid biopolymer of protein and polysaccharide has been fabricated using albumin and chitosan in an effort to reduce the toxicity and augment the biocompatibility of chitosan biopolymers (Karimi et al., 2013).

Dendrimers constitute a highly promising class of organic nanoparticles. They are monodispersed macromolecules with a regularly-shaped highly branched 3D architecture. A dendrimer—regardless of its type, e.g., micellar, chiral—is a branched, highly branched polymer with a spherical shape. It consists of an initiator core, interior layers of polymers, and an exterior functionality layer attached to the outermost interior generations (Malik et al., 2012). Building dendrimers entails repeated addition of carbon and other elements to a central atom such as nitrogen to create spherical branched structures of desired sizes (Gohel et al., 2009). Polyamidoamine (PAMAM) dendrimers are known to have the highest transfection efficiency among other types of dendrimers owing to their large size and the type of their amine groups. In general, primary amine groups participate in DNA binding, thus, promoting DNA cellular uptake. Tertiary amino groups, on the other hand, reinforce the release of DNA into the cytoplasm because they act as a proto-sponge in endosomes (Dufes et al., 2005).

Inorganic Nanoparticles

Inorganic nanoparticles predominantly comprise metals, metal sulfides/oxides, and semiconductors (Sharma et al., 2015). They find numerous applications as gene delivery vectors due to their high surface-to-volume ratio, their optical and magnetic properties, and the convenience of functionalizing them (Ojea-Jiménez et al., 2016).

Metal nanoparticles are colloidal sols of metals such as gold and silver that do not form conglomerates and are not deposited (Helland, 2006). In simple terms, their classic method of preparation involves reduction of metal salts with either sodium citrate or sodium borohydride, and their treatment with polymer-thiol capping layers for stabilization (Peng et al., 2010). Evidence suggests that Romans pioneered the use of metal colloids to dye glasswork and fabrics and to treat arthritis (Rao et al., 2007). However, it was Michael Faraday who, for the first time, observed that gold colloids displayed optical properties different from those of bulk gold (Edwards and Thomas, 2007). Today, the main focus of researchers is on fine-tuning the size and shape, and functionalizing the surface of gold nanoparticles (Giljohann et al., 2010) to realize their full potential in gene therapy (Ding et al., 2014), catalysis (Auffan et al., 2009), ancient DNA detection and analysis (Feuillie et al., 2011; Soleimani et al., 2014), cancer imaging (Kang et al., 2010), among many other applications.

Nanoparticles manufactured with iron oxides, including iron (III) oxide (Fe₃O₄), iron (II) oxide (FeO), and maghemite (Fe₃O₄), form a group of ultrafine, magnetic nanoparticles called superparamagnetic iron oxide nanoparticles (SPION) (Mody et al., 2010). These promising inorganic nanoparticles are synthesized via different methods, including thermal decomposition, co-precipitation (Guivar et al., 2014), hydrothermal synthesis (Ge et al., 2009), and bacterial synthesis (Bharde et al., 2008). Co-precipitation, being the most
common method, involves synthesis of magnetite by dropwise addition of a mixture of ferric chloride, ferrous chloride, hydrochloric acid, and deoxygenated water at a basic pH to sodium hydroxide. The resulting black colloids can then be separated using a magnet. Iron (III) oxide, which is far more useful for biomedical applications, can be synthesized by oxidizing the magnetite colloids at 80°C in an acidic solution (Guivar et al., 2014). These nanoparticles are oftentimes surface-functionalized in order to prevent their aggregation. In addition, nanoparticles with specialized coatings can be attached to certain drugs, genes, or antibodies, and directed to specific sites for delivery (Wu et al., 2008). Surface functionalization employs three types of materials: inorganic molecules (e.g., silica), organic molecules (e.g., fatty acids), and surfactants (e.g., Sodium Oleate) (Andrade et al., 2011). Although the use of each of these materials is accompanied by some challenges, surface-functionalized SPIONs hold great potential as a delivery system in gene therapy; should the issues related to their toxicity, degradability, and elimination be taken care of.

Semiconductor-based nanoparticles are small nanocrystals with a diameter in the range of 1-100 nm (Michalet et al., 2005). Quantum dots are the most widely used semiconductor nanoparticles. They are characterized as fluorescent, colloidal, wavelength tunable nanoparticles. One can easily control their size and shape by adjusting the temperature and duration of synthesis, and the type of ligand molecules used (Michalet et al., 2005). Generally, quantum dots consist of a core and a shell that protects the nanocrystal against oxidation. CdSe/ZnS is a common core-shell quantum dot composed of a cadmium selenide core and a zinc sulfide shell. After synthesis, CdSe/ZnS can be solubilized by ligand exchange and biofunctionalized to serve various biomedical applications (Early et al., 2010). It has been shown that nucleic acids can be conjugated to functionalized quantum dots both covalently and electrostatically (Drbohlavova et al., 2009). Aside from acting as vectors, quantum dots can be used to trace the distribution of nucleic acids in transfected cells, which strengthens their prospects as a practical tool in gene therapy.

Applications in Gene Therapy

Nanoparticles are favorable candidates as gene carriers on account of their nanometric size, high surface to volume ratio, and stability. Furthermore, they can undergo surface modifications in order to bind a boundless spectrum of ligands and receptors. Alternatively, some nanoparticles are able to encapsulate nucleic acids and release them inside target cells. These characteristic features, in addition to the lack of immunogenicity, make nanoparticles an ideal choice as non-viral vectors in gene therapy. This review will discuss successful attempts at using liposomes, chitosan-based nanoparticles, dendrimers, and gold nanoparticles in gene delivery.

Soon after they were described by Alec Bangham in 1965, liposomes have been under scrutiny for use as a delivery system for both drugs and DNA (Sharma et al., 2015). As a vector in gene therapy, liposomes have been employed to tackle corneal diseases (Jun and Larkin, 2003), cystic fibrosis (Caplen et al., 1994), cardiovascular diseases (DzAu et al., 1996), cancer (Balazs and Godbey, 2010), among others. Lung cancer is the most aggressive type of cancer with a relatively poor survival rate, despite the availability of various modes of therapy. In a study conducted by Ito et al., DOTAP: cholesterol liposomes were used to deliver FUS1—a tumor suppressor gene—to lung cancer cells in vivo (Ito et al., 2004). Liposome-DNA complexes were synthesized by simply by mixing a 20 mM DOTAP: cholesterol solution and a solution of FUS1 in 5% dextrose in water. The efficacy of the complex was assessed in both inhibiting tumor growth as well as in preventing metastasis. The results of intratumoral injection of the liposome-DNA complex into subcutaneous tumor cells evidently indicated a meaningful decrease in tumor size. Immunohistochemical analysis further verified the link between tumor inhibition and the liposome-DNA injection, as the FUS1 protein was primarily detected in the tumor cells. Moreover, the complex appeared to be capable of inhibiting metastasis, as demonstrated by the reduced number of tumors in the mice injected intravenously with the complex. The mean survival time of these mice was also shown to be nearly twice as long as those administered with either FUS1 or the liposome alone.

Chitosans were first used as a self-aggregating complex in gene therapy in vitro in 1995 (Tiyaboonchai, 2003). The reason underlying the popularity of chitosans in gene delivery is the fact that the primary amine backbone in chitosans gets protonated (i.e., positively charged) in acidic conditions, rendering the molecule soluble in organic solvents (Raftery et al., 2013). There have been numerous successful studies reporting the therapeutic use of chitosan both in vitro (Jreyssaty et al., 2012) and in vivo (Kittur et al., 2003), not to mention the efforts to investigate the possibility of oral administration of chitosan-DNA complexes (Bowman et al., 2008). Niu et al. reported successful gastrointestinal administration of chitosan-insulin gene complexes to diabetic rats (Niu et al., 2008). In this study, plasmid DNA containing the human insulin gene, after extraction, purification, and heat shock, was mixed with a diluted solution of chitosan in convolution for 1 minute. The complex was given to Wistar rats through lavage and cololysis. The expression of the human insulin gene was confirmed in the models treated via lavage and cololysis through
Nanoparticles in Gene Therapy

RT-PCR and Western Blot analysis. Further, there was a dramatic decline in the level of fasting blood sugar of the rats injected with chitosan-DNA particles, compared to those given either naked chitosan or saline solution alone. In short, the study demonstrated successful transfection and efficient expression of the human insulin gene for the treatment of diabetes.

Inspired by the shape of fungal hyphae and tree roots, dendrimers were first conceptualized and synthesized by Flory et al. in the late 70s (Tomalia and Fréchet, 2002). Dendrimers have enjoyed a plethora of applications over the years, including drug and gene delivery due to their unique properties. The first attempts at using dendrimers as a gene delivery vector centered on PAMAM dendrimers consisting of a core of either ammonia or ethylenediamine with a few branching points (Dufes et al., 2005). In 2004, Marano and colleagues performed the first clinical studies on the application of dendrimers in gene therapy (Marano et al., 2004). The authors made use of synthetic dendrimers complexed with a sense oligonucleotide—displaying anti-human and rat vascular endothelial growth factor (VEGF) activity—in order to inhibit uncontrolled formation of new choriald blood vessels through silencing the VEGF operon. In a more recent effort, Huang et al. demonstrated the efficacy of dendrimers for the long-term treatment of Parkinson’s disease (PD) (Huang et al., 2010). First, dendrigraft poly-L-lysine polyethylene glycol-angiopet (DPA) nanoparticles (belonging to the family of dendrimers) were initially mixed with plasmid DNA, carrying the therapeutic gene GDNF (glial cell neurotrophic factor), and later incubated with brain capillary endothelial cells (BCECs). Second, the PD models were injected intraperitoneally with GDNF-DPA, followed by behavioral and immunohistochemical tests. The former involved evaluating the behavior of the models based on line crossing, rearing, head dipping, defecating, and inactive sitting. The models treated with GDNF-DPA showed considerable improvements in the number of line crossings and inactive time. Moreover, the results of immunohistochemistry indicated that multiple doses of GDNF-DPA reduced the loss of dopaminergic neurons in the PD models, further reinforcing the viability of dendrimers as effective, non-viral gene vectors.

Gold nanoparticles have also been demonstrated to provide a reliable delivery system for gene therapy. Their attractive properties such as inertness, ease of functionalization with thiol linkages, and plasmon resonance have spawned extensive research to explore their therapeutic potential (Ghosh et al., 2008). One of the earliest studies to assess gold nanoparticles as nucleic acid vectors was accomplished by McIntosh et al. who successfully inhibited the transcription of T7 RNA polymerase using gold nanoparticles functionalized with trimethylammonium thiol (McIntosh et al., 2001). Other technologies can be used in conjunction with gold nanoparticles to mediate selective release of nucleic acids or increase their transfection efficiency. For instance, Wijaya et al. took advantage of the photo-physical properties of gold nanoparticles to selectively release two different DNA conjugates from two different sets of gold nanorods (Grabinski et al., 2011). Briefly, each nanorod melted by near infrared irradiation at its plasmon resonance peak, releasing its DNA molecule. In another study, Heinemann and colleagues used a technique called gold nanoparticle mediated (GNOME) laser transfection to improve the transfection efficiency of gold nanoparticles (Heinemann et al., 2013). The technique involved irradiation of cell membrane-bound gold nanoparticles, which activated their plasmon resonances, causing perforations in the membrane, which led to diffusion of the nanoparticles into the cell. Cells were transfected with enhanced green fluorescent protein (EGFP) and later laser-transfected with anti-GFP siRNA (small interfering RNA). Flow cytometry and western blot analysis showed a higher decrease in the expression of EGFP in the laser-transfected cells in comparison with those transfected without irradiation.

Potential Hazards and Ethical Aspects of Nanoparticles

Nanoparticles have the potential to cause many different pathologies of the gastrointestinal, respiratory, and cardiovascular systems. An experiment done on mice by intra-tracheal instillation of carbon nanotube particles showed that the carbon nanotube itself has the ability to cause many lung pathologies to mice such as peribronchial inflammation, interstitial inflammation, epithelioid granuloma, and necrosis of lungs (Warheit et al., 2003). In the same study, the toxicity produced by carbon nanotubes was much greater compared to the toxicity caused by carbon black and quartz (Warheit et al., 2003). Another potential hazard is that nanoparticles can enter the human body through many ports, whether it is accidental or not, they can enter the lungs, and the central nervous system either through systemic circulation through the ollfactory bulb, or directly through axons of olfactory pathway, which can also cause inflammatory reactions in the brain (Seaton et al., 2009). Inflammatory bowel diseases can also happen if the nanoparticles are extrapolated to the gastrointestinal system. The ability of nanoparticles to induce release of pro-inflammatory mediators is highly related to their toxicity, and this can result in inflammatory response and therefore organ damage (Hristozov and Malsch, 2009). Furthermore, if they are ingested, they can reach the circulation and afterwards reach different systems of the body and different organs, which might result in toxicity (Yildirimer et al., 2011). Ethical and law issues should be taken into consideration since the science of Nano-biotechnology is rapidly expanding. It is important to consider those
issues in order to stop or decrease its side effects and potential hazards on the public health and environment. The ethical considerations involved in Nano-biotechnology are related to risk assessment in general, germline-cell vs. somatic-cell therapy, managing the risk of engineered nanoparticles, enhancing human capabilities using nanoparticles, uncontrolled self-assembly and function of nanoparticles, research into human embryonic cells, and the toxicity of nanomaterials (Matsuura et al., 2009). Toxic effects of nanoparticles on health in general is very serious, nanoparticles may cause dangerous effects due to their very small size which allows them to be highly mobile in the environment and the human body (Becker et al., 2007). Nanoparticles can enter human tissues through many different ports such as through the digestive system, through the lungs by accidentally inhaling them, and even through the skin. Inhaling the nanoparticles may cause systemic distribution of those particles; they can reach the blood brain barrier, to reach afterwards the olfactory bulb and the cerebellum (Yang et al., 2008). Another concern is that the materials which those nanoparticles are made up of, many of them are made of non-biodegradable pollutants, such as metals and carbon black, the long-term behavior of such materials should be considered because it is still unknown (Shin and Jang, 2007). However, some nanoparticles are made up of silver, it is known that it has some toxic effects on humans and may enter the body through many different portals, silver can cause some early changes in the cell membrane’s permeability to potassium and then to sodium at concentrations that don’t limit the activity of Na⁺, K⁺-ATP. Silver nanoparticles can also cause toxic effects on peripheral blood mononuclear cells (PBMCs), the proliferation and the cytokine expression by PBMCs. Cadmium is a heavy metal used in nanoparticles, but it is often recognized as a toxinant, because it has the ability to cause lysosomal damage and DNA breakage in the hepatocytes of mammals. Cadmium can also damage the mitochondrial function and cause apoptosis (Rzgalinski and Strobl, 2009). Ethical considerations of Nano-biotechnology also should include the non-medical applications, such as the human enhancement. Since the nanoparticles can be used to diagnose, prevent, or treat diseases, they can also be used to enhance the appearance or the function of the human body, or even the human mind, which gives rise to an important ethical issue, which is using nanoparticles in physical enhancement instead of treatment (Mehlman, 1999). For example, anabolic steroids can be prescribed to help patients recover from traumatic injuries; however, athletes may use these drugs to improve their performance. Another issue is that the applications of Nano-biotechnology in the field of neurology can be achieved by using special nanoparticles to help reduce or replace memory loss, which on the other hand, can be used to enhance the normal human memory. Moreover, nanoparticles or nanomedicines which are specifically engineered to help people who have some learning disabilities may also be used by normal and healthy people to become super-intelligent (Khushf, 2008).

Conflict of interest
The authors declare no conflict of interest with respect to the content and writing of the paper.

References


Nanoparticles in Gene Therapy


Kowalczyk T, Hnatuszko-Konka K, Gerszberg A, Kononowicz AK (2014) Elastin-like polypeptides as a promising family of genetically-
Nanoparticles in Gene Therapy


Nanoparticles in Gene Therapy


