Nanomedicine and Drug Delivery Strategies for Treatment of Genetic Diseases

Janet Hsu[1] and Silvia Muro[1,2]
[1]Fischell Department of Bioengineering
[2]Institute for Bioscience and Biotechnology Research
University of Maryland, College Park
United States of America

1. Introduction

Effective diagnostic and therapeutic intervention of genetic diseases have been particularly pending on technological development. It was not until the last two decades of the past century that molecular biology and recombinant DNA have provided diagnostic and therapeutic tools for these diseases. Since then, our understanding of the biological processes underscoring genetic conditions and the development of technologies enabling their study and treatment have vastly grown, providing scientists and clinicians with means to continue advancing in these directions. Recently, another technology has strongly impacted the fields of molecular biology, medicine, diagnostic and therapeutic development: nanotechnology.

More precisely, nanotechnology focuses on the study and manipulation of matter and processes at the nanometer (10^{-9} m) scale, encompassing the atomic, molecular, and macromolecular scales. In this chapter, we use the term nanotechnology from a more broad perspective, referring to devices fabricated at the sub-micrometer scale, a size compatible with that of large biological macromolecular complexes and cellular organelles, which are often referred to as nano- as well, due to the nanoscale of their architectural and/or functional components. The application of nanotechnology principles and tools to the field of medicine, known as nanomedicine, is also a relatively young field that has vastly expanded within the last couple of decades to render new diagnostic and treatment approaches. However, delivery of active agents assisted by these technologies is still a relatively unexplored strategy in the case of genetic diseases.

This chapter discusses some of the technological advances regarding the application of nanomedicine to the treatment of genetic conditions. Some basic foundation on the design and features of these technologies are provided, including targeting, transport, and delivery capabilities of drug delivery systems. Applications in the context of genetic diseases are also discussed, including treatment of phenotypic symptoms and complications associated to these conditions, and correction therapies, such as treatment with small molecules, non-viral gene therapies, cell therapies, and enzyme replacement therapies.

2. Types and properties of drug delivery tools used in nanomedicine

An area of research where nanotechnology and nanomedicine applications have been particularly prolific pertains to delivery of diagnostic and therapeutic agents. Drug delivery

carriers are macromolecular assemblies that can incorporate imaging and therapeutic compounds of distinct nature, such as small chemicals, fluorophores and biosensors, peptides and proteins, and oligonucleotides and genes. They can be designed to improve the solubility of these cargo molecules and their bioavailability, and also to control their circulation, biodistribution in the body, and release rate, altogether enhancing their efficacy (Langer, 1998, Duncan, 2003, Panyam & Labhasetwar, 2003, Moghimi, et al., 2005).

2.1 Nanocarrier design

A broad spectrum of materials (biological, synthetic and semi-synthetic) assembled in a variety of conformational arrays (from linear structures to branched and dendritic counterparts, micelles, hollow capsules, porous or solid particles, etc.) have been designed to help in diagnostic and therapeutic interventions (Langer, 1998, Discher & Eisenberg, 2002). Examples of these include carbon nanostructures, quantum dots, metal particles, liposomes, and formulations based on natural and/or synthetic polymers (Figure 1). The composition and architecture of these systems play an important role in determining their translational capabilities, including their ability to carry cargoes of different chemistries and their loading capacity, stability, biodegradability and overall biocompatibility, and various functional aspects (Moghimi, et al., 2001, El-Sayed, et al., 2005, Stayton, et al., 2005, Torchilin, 2006).
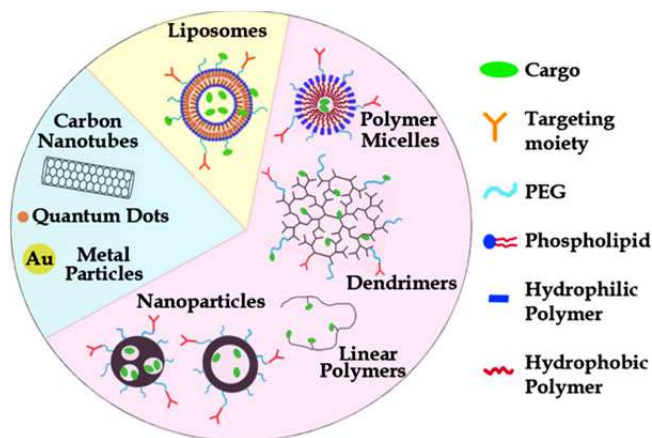

Fig. 1. Composition and architecture of carriers and other assemblies (not drawn to scale) utilized in nanomedicine for diagnosis, imaging, and drug delivery. Yellow, purple, and blue colors correspond to formulations discussed in sections 2.1.1, 2.1.2, and 2.1.3.

2.1.1 Lipid vesicles

Lipid-based micelles (encapsulating vesicles formed by a single phospholipid layer, ranging from ~5 to 100 nm in diameter) and mostly liposomes (vesicles delimited by a phospholipid bilayer, with sizes from 50 nm to several micrometers) have been extensively studied for more than half a century in the context of transport of diagnostic and therapeutic agents (Musacchio & Torchilin, 2011). These vesicles can be constructed with natural lipids, mainly derivatives of phosphatidyletanolamine, phosphatidylcholine, phosphatidylserine, and phosphatidylglycerol, to mimic cellular membranes. Because phospholipids can spontaneously form lipid bilayer-contained capsules in aqueous solution, hydrophilic

molecules can be loaded within the inner volume of the vesicle or attached on the liposome surface, while hydrophobic cargo can be embedded within the bilayer. Targeting moieties with affinity to markers in the body, imaging molecules, and/or polymer coats (discussed below) can be incorporated on the surface in order to enhance their circulation, provide selective targeting, and improve transport (Minko, et al., 2006, Musacchio & Torchilin, 2011).

Yet, liposomes can only bear a relatively low degree of said modifications, rendering them relatively unstable and susceptible for rapid clearance, mainly due to deposition of plasma immunoglobulins and proteins on their surface, favoring uptake by macrophages of the reticuloendothelial system (Moghimi & Szebeni, 2003). New approaches are combining polymers with liposomal formulations to improve these aspects (Musacchio & Torchilin, 2011).

2.1.2 Polymer nanocarriers

Polymers are macromolecules that result from covalent linkage of smaller structural units or repeats referred to as monomers, and can be designed from fully synthetic to biological blocks (Moghimi, et al., 2001). Some examples of synthetic polymers employed in drug delivery include chemically inert and non-toxic materials such as poly(methyl methacrylate), poly(acrylic acid), polyacrylamide, poly(vinyl alcohol), and poly(ethylene glycol), among many others. Biological polymers include (but are not restricted to) natural gums, polysaccharide-, and polypeptide-based formulations (e.g., chitosan, sodium alginate, gelatin, albumin, etc.). Also commonly used due to their biocompatibility and ability to be degraded in the body are polyanhydrides, polyorthoesters, polycaprolactone, polylactides, polyglycolides, and poly(lactide-co-glycolides). These carriers encompass linear polymers, branched polymers and tree-like hyperbranched dendrimers, self-assembled polymersomes (the polymer counterpart of liposomes), and more stable particles, with overall sizes ranging from a few nanometers to several micrometers (Torchilin, 2006). They can endure a degree of modification higher than that of liposososmes, rendering multifunctional formulations that can carry therapeutics, targeting moieties, and imaging agents (Duncan, 2003).

Among them, the polyether poly(ethylene glycol), a polymer of ethylene oxide also known as PEG (Figure 2B in section 2.2.1), is one of the polymers most broadly used in nanomedicine due to its high hydrophilicity and, hence, its ability to solubilize hydrophobic drugs, minimize interaction with the immune system, and prolong the circulation of compounds (Moghimi, et al., 2001, Panyam & Labhasetwar, 2003). PEG can be directly linked to the therapeutic cargo of interest (oligonucleotides or DNA, proteins, small molecules, etc), or it can be coupled to or grafted onto other carriers (Moghimi, et al., 2003).

Poly(lactide-co-glycolide), known as PLGA, is an FDA-approved material that is also commonly used for drug delivery due to its biocompatibility: PLGA is hydrolyzed into lactic and glycolic acids with minimal toxicity (Mundargi, et al., 2008) and the rate of degradation can be adjusted by modification of the particle size and the lactide-to-glycolide ratio for controlled release of the cargo (Panyam & Labhasetwar, 2003). In addition, endo-lysosomal escape of PLGA carriers can be regulated to some extent by modifying these design parameters (Panyam, et al., 2002), which is useful for gaining access to other cell compartments. This is also a property of cationic polymers, such as poly-L-lysine and poly(ethylenimine) (PEI), whose amine groups become protonated at endosomal pH, resulting in osmotic swelling that bursts the endosome (Sonawane, et al., 2003). Although these polymers are useful for transfection in cell cultures, due to toxicity, other experimental formulations more amenable for *in vivo* applications are being explored. This is the case for pH-sensitive poly(acrylic acid) derivative carriers that act as proton sponges within

lysosomes, and temperature-responsive polyelectrolyte hydrogels whose hydration rate varies with temperature, leading to changes in the carrier volume within the endo-lysosomal compartment (Yessine & Leroux, 2004, El-Sayed, et al., 2005, Choi, et al., 2006).

Also natural polymers, such as chitosan, are often exploited for their pH sensitivity and bioadhesive properties. Chitosan is cationic under neutral or basic conditions, favoring adhesion to negatively-charged membranes of cells, and is depolymerized under acidic conditions, resulting in pH controlled drug release (Agnihotri, et al., 2004). Since chitosan is mucoadhesive and favors permeabilization through the gastrointestinal epithelial layer, this polymer is particularly useful to deliver agents by the oral route (Agnihotri, et al., 2004). Polymer dendrimers are also suitable for transport across the intestine due to their small size and ability to enhance permeability of the epithelial layer (Kitchens, et al., 2005).

2.1.3 Carbon nanostructures, metal particles and quantum dots

In addition to liposomes and (synthetic or natural) polymer carriers, carbon nanostructures, quantum dots, and metal particles are becoming more commonly used in nanomedicine. These materials are often employed in the design of biosensors for diagnostic applications, and also some derivatives have been studied in the context of drug delivery.

Carbon nanostructures (~1-3 nm diameter, (Chen, et al., 2003)), such as single- or multi-walled carbon nanotubes, carbon nanospheres, extended carbon nanocube networks, etc., possess interesting physical properties suitable for applications in nanotechnology, electronics, and optics. They are highly stable and versatile in terms of their structural conformation, charge, strength, flexibility, etc. (Cheung, et al., 2010). This material can also be functionalized with drugs and/or biomolecules for diagnostic and therapeutic purposes.

Quantum dots (QDs) are semiconductors fabricated from a combination of metals and non-metals (Choi & Frangioni, 2010). These nanoparticles (~2-10 nm diameter) have unique photophysical properties, e.g., upon excitation they emit fluorescence that is brighter and more stable than that of traditional fluorophores, and their size can be varied to achieve excitation and emission at different wavelengths. They can be detected with high sensitivity, which along with their stability, provides an ideal ground for the design of *ex vivo* screening technologies, such as the case of effective diagnosis of genetic diseases (Bailey, et al., 2009).

Metal nanoparticles are also used in biosensors due to their charge, high sensitivity, and output stability. Gold is often used for imaging and biosensing due to its biocompatibility and unique optical sensitivity (Cai, et al., 2008). Phosphor particles contain rare earth metals and have similar characteristics as QDs, being highly photostable and rendering virtually no autofluorescence, yet their larger size limits their applications (Corstjens, et al., 2005).

While the formulations described above are mostly employed for *ex vivo* diagnostic applications due to toxicity, iron oxide nanoparticles, preferentially 10-100 nm in size (Shubayev, et al., 2009), which can be additionally coupled to polymers, are being explored for imaging and drug delivery. These carriers can be functionalized with various cargoes and have been shown to be relatively suitable for transport of therapeutics (Shubayev, et al., 2009). They present low toxicity, being biocompatible for *in vivo* applications. Iron oxide and, particularly, superparamagnetic iron oxide (SPIO) nanoparticles exhibit magnetic properties, which is used for MRI imaging (Kamaly & Miller, 2010) and also provides an opportunity to control particle transport by external magnets (Nacev, et al., 2010).

2.2 Pharmacokinetics, targeting, and sub-cellular transport of nanocarriers

Development of effective diagnostic techniques and therapeutic delivery methods carry a number of important challenges, many of which may be overcome with the input of nanomedicine approaches. The size scale of nanomedicines and the degree of manipulation to which they can be subjected, make these endeavors seemingly tangible. Drug delivery carriers can be functionalized to improve control of their circulation and biodistribution in the body at the tissue, cellular, and sub-cellular level. This can be achieved by incorporating immune-evading moieties and/or affinity molecules that favor adhesion to either general or specific biological markers, depending on the degree of selectivity required. In addition, when carriers are targeted to cellular receptors involved in endocytic transport or coupled to cell penetrating peptides, or if they are designed to modify the permeability of cellular barriers, they also provide delivery to a variety of intracellular compartments, such as the lysosome, cytosol, nuclei, etc., and can furthermore be transported at some extent across cellular layers, a requirement for a number of clinical goals (Langer, 1998, Moghimi, et al., 2001, Panyam & Labhasetwar, 2003, Sahay, et al., 2010, Sawant & Torchilin, 2010).

2.2.1 Circulation and clearance

When administered *in vivo*, therapeutic agents are often recognized as foreign substances and, consequently, are rapidly cleared from the body (Moghimi, et al., 2001, Yoo, et al., 2010). This is a general obstacle of classical means of drug delivery that also applies to chemicals used as palliative treatment of symptoms associated to genetic diseases, and more specific small molecules used to regulate affected metabolic pathways, inhibitors and activators of the affected molecules, chaperones to improve folding and stability, and recombinant proteins and enzymes used for replacement therapies. Clearance of foreign compounds in the body occurs mainly by the reticuloendothelial system and other elements of the immune system, as well as by renal filtration (Moghimi & Szebeni, 2003). Resident macrophages in the alveoli remove substances administered into the lungs through the respiratory tract, Kupffer's cells in the liver sinusoids remove materials that enter the portal circulation through the gastrointestinal epithelium, materials administered in the systemic circulation are cleared mainly by the spleen and liver, and the lymph nodes remove substances that arrive to the tissue parenchyma by draining them through the lymphatics.

For most applications, including those designed for treatment of genetic conditions, e.g., mostly applicable to small molecule therapies and enzyme replacement therapies, rapid clearance is detrimental as it minimizes the chances of the delivered agent to reach its targets in the body and accumulate there at amounts amenable to render significant efficacy. Avoiding recognition by said clearance systems, e.g., by designing immune evasive strategies leading to lengthening of the life-span of the cargo, can improve the efficiency.

One strategy to control this parameter consists of coupling to poly(ethylene glycol) or PEG, known as PEGylation or stealth technology. PEG helps form a hydrophilic brush around cargoes and/or their carriers, minimizing interactions with plasma opsonins, the complement, professional phagocytes, and lymphocytes which provide specific immunity (Moghimi & Szebeni, 2003) (Figure 2A and 2B). As a consequence, certain physiochemical properties of the cargo (such as hydrophobicity) are altered, allowing the platform to gain solubility and to remain elusive from immune detection. This prolongs the circulation in the bloodstream from a few hours to days, which favors lengthened medicinal effects and less frequent administrations (Moghimi & Szebeni, 2003, Musacchio & Torchilin, 2011).
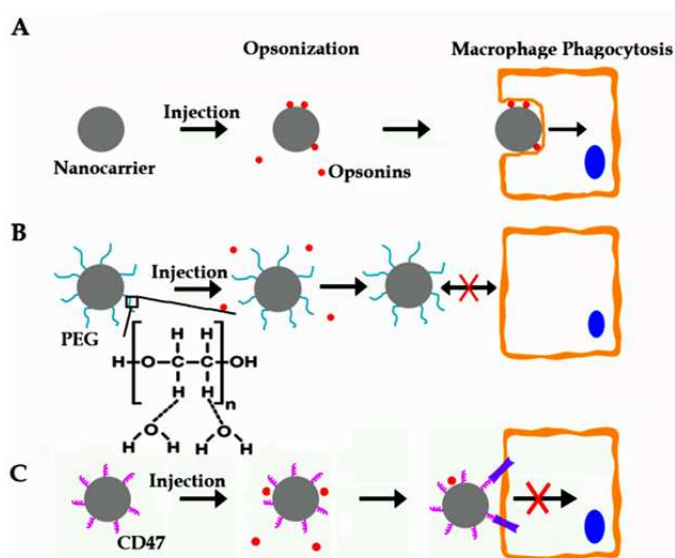
Fig. 2. Strategies to minimize rapid clearance of nanocarriers. (A) Poly(ethylene glycol) or PEG minimizes interaction with plasma proteins and binding to macrophages. (B) CD47 binding to the surface of immune cells inhibits engulfment and phagocytosis.

Other strategy to minimize drug removal takes advantage of the natural mechanism by which red blood cells in the body avoid clearance by elements of the innate immune system. This is the case for CD47 (Figure 2C), a transmembrane protein that acts like a marker of the "self" by binding to its cognate receptor expressed on leukocytes, inhibitory receptor signal regulatory protein alpha (SIRPα). CD47 binding to SIRPα inhibits phagocytosis, in part via regulation of the cytoskeleton and inhibition of engulfing structures. Incorporation of CD47 on drug carrier surfaces reduces attachment to neutrophils and macrophages, therefore prolonging circulation and inhibiting inflammation (Stachelek, et al., 2011).

In addition to controlling the solubility level, half-life in circulation, and immune system recognition, nanocarriers can also improve control of the drug efficacy upon release in the case of therapeutic interventions where administration is local. Localized implantation of bioactive agents embedded within porous matrices and/or hydrogels capable of responding to microenvironment properties can provide controlled release and effects (Tokarev & Minko, 2010). Encapsulation within these formulations can also provide sustained release over prolonged periods of time, as oppose to bulk delivery of a naked therapeutic (Tokarev & Minko, 2010), which can apply to the release of encapsulated drugs and also bioactive substances produced by cells encapsulated within these matrices (discussed in section 3.4).

2.2.2 Targeting

As described above, nanomedicines can improve the bioavailability and pharmacokinetics of diagnostic and therapeutic agents, also protecting them from rapid degradation. In order to maximize their efficacy, carriers can also be designed to help maximize bioadhesion to areas in the body where their action is required, a strategy known as targeting (Figure 3).
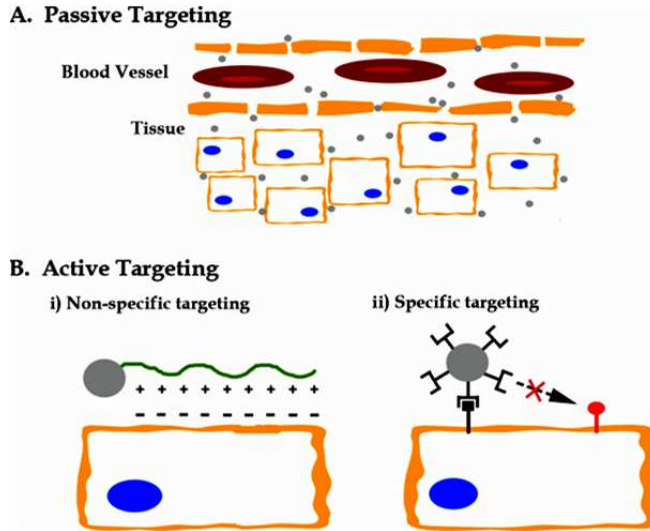
Fig. 3. Passive (A) and active (B) targeting of drug carriers helps localize their cargo within the body, either via non-specific interactions with the target cells (i) or more specific recognition of particular surface markers (ii).

In some cases, general enhanced delivery throughout the body, rather than specific delivery to particular organs, is preferred. This is the case for genetic conditions that affect multi-organ systems due to ubiquitous distribution of the molecular markers or functions affected, such as in many monogenic disorders with both peripheral and central nervous system components. Since most therapeutics do not present intrinsic affinity to cells in the body, coupling them to carriers with affinity properties provides advantages. Hydrophilic and slightly positively-charged polymers provide affinity to the negatively-charged plasma membrane of cells (Panyam & Labhasetwar, 2003, El-Sayed, et al., 2005). Also, these systems can be coupled to affinity moieties that enhance bioadhesion. This is the case for promiscuous affinity peptides such as Tat, antennapedia, and other sequences that gain access to the plasma membrane of cells due to their positively-charged nature (Suk, et al., 2006, Sawant & Torchilin, 2010) (Figure 3Bi), similarly to polymer carriers described above.

In other cases, more specific localization of drug cargoes and imaging agents is necessary for optimal effects, such as the case of diseases with more prominent symptoms in particular organs or cell types (Langer, 1998, Torchilin, 2006, Pardridge, 2010). Moreover, such specific targeting may also reduce potential side effects of the therapeutic in non-intended targets. For instance, carriers injected in the circulation are passively targeted to organs irrigated by the vascular bed immediately downstream the area of administration (first pass phenomenon), such as the case of pulmonary accumulation of carriers administered intravenously. Also, nanocarriers can gain preferential access to organs irrigated by discontinuous blood vessels (Figure 3A), which do not pose a barrier from free diffusion of substances between the circulation and tissue, such as in the liver, an organ considered a main therapeutic target for many monogenic diseases that affect metabolic pathways.

However, delivery of therapeutics to most other sites in the body requires more complex and precise strategies of active targeting. This can be achieved by coupling to affinity

moieties that recognize specific markers expressed by the cells which require intervention (Figure 3Bii), including natural ligands of such markers, proteins and peptides, antibodies and their fragments, sugars, and aptamers. Also, targeting to markers that are expressed under certain pathological processes (as opposed to control physiological conditions) helps favoring delivery to disease sites (Muro & Muzykantov, 2005, Bareford & Swaan, 2007).

Whether it is due to targeting via positively-charged moieties or by specific affinity means, targeted delivery of drug carriers offers advantages over direct targeting of therapeutics. Apart from the described advantages posed by increased solubility, circulation time, and release control, carriers bearing multiple copies of an affinity moiety display greater affinity due to this multivalency, compared to drugs that are directly coupled to one copy of the same affinity molecule (Muro, et al., 2006a). As described below, multivalency of targeted carriers also provides tight binding to cell surface receptors, which can favor uptake within the cell, a necessary requirement for many diagnostic and therapeutic applications.

2.2.3 Sub-cellular transport

For most applications the molecular targets for intervention are intracellular (Langer, 1998, Torchilin, 2006). Therefore, targeting to selected cells and tissues is not sufficient to attain significant effects, the delivered cargoes must also gain access to intracellular compartments where their molecular targets are located. Interventions related to RNA interference or delivery of antisense oligonucleotides require transport of these cargoes to the cytosol of the cell, where the target is accessible. This is also the case for delivery of some chaperones, inhibitors or activators, enzymes and other proteins located in the cytosol or sub-cellular organelles such as the mitochondria, peroxisomes, etc., which can be re-directed to these compartments by signal peptides if delivered previously to the cytosol. Gene therapies also require delivery to the cytosol, with subsequent transport to the cell nucleus. In all these cases, cytosolic delivery can be granted mainly by two routes: direct transport of the cargoes and/or their carriers from the extracellular space to the cytosol of cells, or engulfment by the plasma membrane and uptake into vesicular compartments (Muro & Muzykantov, 2005, Suk, et al., 2006, Bareford & Swaan, 2007, Sahay, et al., 2010).

Several strategies have been designed to directly overcome the plasmalemma, gaining access to the cytosol. These include physical means such as electroporation and ultrasound, where a local electric or ultrasound pulse is exerted in the immediate post-administration period, causing transient enhancement of the plasmalemma permeability (Trollet, et al., 2008), and biolistic particle delivery systems, where penetration into cells is gained by means of tungsten or gold particles that are propelled by a "gene gun" across the plasma membrane (O'Brien, et al., 2001). Other methods providing a similar outcome relate to polycationic lipids used to assist cell transfection, which can bind negatively charged proteoglycans at the cell surface and favor cytosolic delivery by porating the plasmalemma (Dincer, et al., 2005, Zuhorn, et al., 2005). Positively-charged cell penetrating peptides (such as RGD and Tat peptides) also bind to the cell surface due to electrostatic interactions and can facilitate cytosolic delivery of cargoes (Magzoub, et al., 2005, Suk, et al., 2006).

As opposed to gaining access to the cell interior by direct penetration into the cytosol, uptake within endocytic vesicles and subsequent selective permeabilization of these compartments for cytosolic release is another major area of research regarding the design of precise nanomedicines (Figure 4A). For instance, drug carriers can be targeted to cell surface receptors involved in endocytosis (Muro & Muzykantov, 2005, Bareford & Swaan, 2007, Pardridge, 2010, Sahay, et al., 2010). This term refers to a group of processes by which cells

engulf extracellular material with their plasma membrane, followed by pinching off the resulting vesicles into the cytosol. Uptake by endocytosis is regulated by numerous pathways (clathrin- and caveolar-mediated mechanisms, macropinocytosis, phagocytosis, and other non-classical mechanisms), and most commonly results in transport of the internalized materials to endosomes and lysosomes (Bareford & Swaan, 2007, Sahay, et al., 2010). This strategy is ideal in the case of delivery of therapeutic agents whose action is required at said sub-cellular compartments, such as in the case of carrier-assisted delivery of enzyme replacement for lysosomal storage disorders (Figure 4A right) (Garnacho, et al., 2008, Muro, et al., 2008, Muro, 2010, Hsu, et al., 2011). For those cases where delivery to the cytosol and access to other sub-cellular compartments is required (Figure 4A left), carriers can be coupled to fusogenic peptides derived from bacterial toxins (e.g., hemaglutinnin and GALA peptides), which can induce poration of the endosomal membrane in response to gradual pH lowering in these compartments (Kakudo, et al., 2004). Carriers themselves can also be designed to overcome endosomal membranes, such as in the case of pH-sensitive poly(acid) carriers and temperature-responsive poly(electrolyte) hydrogels (Yessine & Leroux, 2004, Stayton, et al., 2005, Choi, et al., 2006, Oishi, et al., 2006).
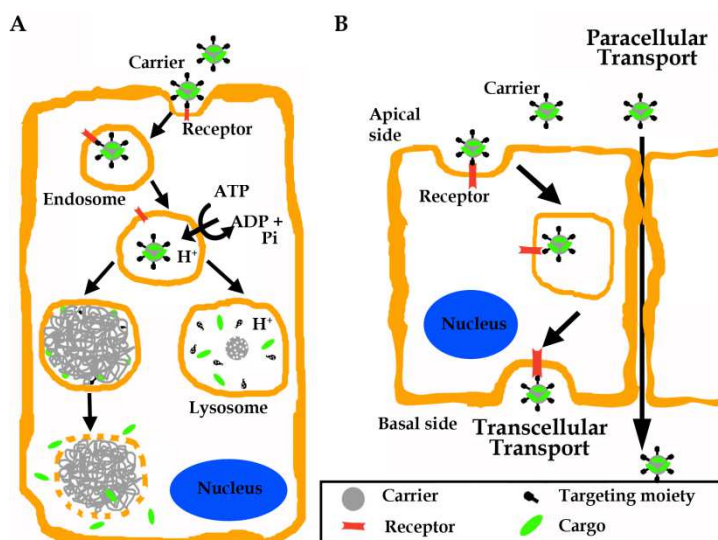

Fig. 4. Sub-cellular delivery of nanocarriers. (A) Endocytosis of carriers can lead to transport to endosomes and lysosomes, where acidic pH and hydrolases can degrade the carrier components and release the therapeutic cargo. Alternatively, pH-sensitive carriers can destabilize the endosome, releasing the cargo to the cytosol. (B) Transport across cellular layers can take place either via paracellular transport between adjacent cells or via transcytosis across the cell body (by vesicular mediated endocytosis and exocytosis).

Finally, effective accumulation within particular tissues requires penetration across cellular barriers. This is most evident in the example of diagnostic and therapeutic agents aimed to exert their activities in the central nervous systems (Pardridge, 2010), and those which need to be transported across the gastrointestinal epithelial layer upon oral administration (Kitchens, et al., 2005, Serra, et al., 2009, Park, et al., 2011). In addition, except for a few cases

of vascular beds characterized by discontinuous endothelium where free diffusion is granted, transport of substances from the bloodstream into the irrigated tissues is regulated by the layer of endothelial cells that separate the microvascular wall from the tissue parenchyma and this poses an obstacle to penetration (Pardridge, 2010). Transport of hydrophilic and/or relatively large substances (molecular chaperones, proteins and enzymes, polysaccharides, and oligo- and poly-nucleotides) across endothelial or epithelial barriers is restricted to the transcellular or paracellular routes (Figure 4B). The transcellular route involves internalization of materials on the apical membrane via endocytosis, traffic of endocytic vesicles across the cell body, and exocytosis at the basolateral membrane (transcytosis; Figure 4B left), which occurs mainly via clathrin- and caveolar-mediated pathways (Muro, et al., 2004, Bareford & Swaan, 2007). Due to its safety, this route is preferred for transport into the central nervous system, which can be achieved to some extent by targeting receptors such as the folate, insulin, low density lipoprotein, and transferrin receptors (Hilgenbrink & Low, 2005, Pardridge, 2010, Musacchio & Torchilin, 2011). The paracellular pathway (Figure 4B right) involves transport between adjacent cells via regulation of the junctions that interlock them (Dejana, 2004). This may lead to more uncontrolled, passive transport compared to the transcellular route, hence it is less desired in gaining access into the brain, whereas it seems relatively safe for transport across the gastrointestinal epithelium in oral applications (Kitchens, et al., 2005, Park, et al., 2011).

3. Nanomedicine applications for treatment of genetic diseases

Several properties of nanomedicine designs, mainly pertaining their biocompatible size and high degree of manipulation that allow adaptation to different biomedical applications, have caused this field to be considered a new technological revolution. Nanotechnology has opened new possibilities for *ex vivo* detection methods (e.g., applicable to mutation screening) as well as biomarkers of disease, with several technologies being also applicable for *in vivo* imaging (Cai, et al., 2003, Corstjens, et al., 2005, Bailey, et al., 2009, Cheung, et al., 2010, Choi & Frangioni, 2010). These strategies are considerably more sensitive than traditional methods, permitting detection in smaller samples and/or providing more accurate measurements and tracings of the parameters of interest. From the therapeutic perspective, nanomedicine strategies hold considerable promise to improve control parameters such as the solubility, stability, clearance, biodistribution, sub-cellular transport, controlled release of therapeutic cargoes of diverse nature, improving their efficacy and minimizing potential side effects (Langer, 1998, Moghimi, et al., 2001, Stayton, et al., 2005, Torchilin, 2006).

For the most part, these technologies are still at the experimental stage, particularly those that require *in vivo* administration as opposed to those designed for *ex vivo* diagnosis and detection. However, research has shown a great potential of these platforms for clinical translation in the near future, with some examples being already available in the market, mostly in the case of cancer therapeutics. Although relatively unexplored in the case of genetic deficiencies, the use of nanomedicine principles and strategies for diagnosis and treatment of these conditions is a rapidly growing field with highly promising perspectives.

3.1 Small molecule therapy

Small molecule therapy typically encompasses chemicals used either for palliative care of symptoms or more specifically designed to cope with a particular landmark that regulates disease progression. In many cases, their small size and chemical properties are relatively

permissive of diffusion through the body and cells, with relatively good efficacy. However, in other cases they suffer from the obstacles discussed in section 2.2 above, including rapid clearance, inactivation, and sub-optimal access through biological barriers.

For instance, when delivered intravenously rapid loss of therapeutic activity may arise from the direct interaction with blood components and elements of the reticuloendothelial or immune systems. This can be prevented by encapsulating small molecule therapeutics within carriers designed to minimize interaction with said clearance or inactivating systems, prolong their circulation, enhance their accumulation in certain areas of the body, and control their release over long periods of time. Some examples of such applications in the realm of intravenous administration of small molecule therapies for genetic conditions have been reported, such as the case of treatment of hemophilia with coagulation factors encapsulated in biocompatible liposomes, which prolongs the therapeutic window (Yatuv, et al., 2010), or enhanced efficacy of liposomal formulations of antibiotics to treat lung infections in cystic fibrosis (CF) (Mugabe, et al., 2005, Rukholm, et al., 2006). In other cases, interaction with blood components is desired, yet formulation as a nanocarrier still provides therapeutic advantages, such as the case of hexadentate-terminated hydroxypyridinone-based dendrimers, an iron chelating formulation that has been shown to effectively sequester excess iron in haemochromatosis and the thalassaemias (Zhou, et al., 2006).

Regarding penetration in the body, nanomedicine formulations can also help by providing means to modulate viscosity of physiological barriers, such as the sputum in the lungs in the case of inhaled drugs, or enhance the permeability of cellular barriers, such as that encountered by oral drugs in the gastrointestinal epithelium or small molecules that can not cross from the bloodstream into the brain. For instance, the thick lung sputum encountered in CF patients is a major obstacle for effective penetration of classical inhaled therapeutics, hindering access to the underlining epithelial layer. Encapsulation of antibiotics within liposomes modified with amiloride hydrochloride has been shown to force water retention and reduce viscosity of the sputum, resulting in enhanced release and effects (Chougule, et al., 2006). Liposomes modified with the bioadhesive metal, gallium, improved delivery of antibiotics in the CF airways, permitting reduction of the administered dose by an order of magnitude compared to the naked antibiotic (Halwani, et al., 2008). Biodegradable PEGylated PLGA nanoparticles have also been shown to effectively cross the sputum, which has been used to enhance delivery of proteosome inhibitors in CF (Vij, et al., 2010).

With regard to delivery of small molecules by the oral route (preferred to minimize cost and improve patient compliance), encapsulation in enteric-coated capsules is typically required. Classically, bulk capsules protect therapeutics from exposure to the acidic environment in the stomach. Coupling to nanocarriers has shown advantages in this arena, particularly regarding intestinal absorption. This has been shown in the case of oral delivery of anti-diabetic agents by solid lipid particles, which effectively decreased glucose in circulation (Nnamani, et al., 2010) or the case of PLGA nanocarriers encapsulating curcumin for CF, which surpassed the effects of its non-encapsulated counterpart (Cartiera, et al., 2010). Similar results were rendered using chitosan nanocarriers designed for pH-sensitive release and enhanced mucoadhesive potential, resulting in improved absorption of anti-diabetic drugs (Wong, 2010). A combination of classical oral delivery formulations and nanotechnologies, e.g., chitosan nanocarriers encased within enteric-coated capsules for insulin delivery, has shown enhanced results, reflecting the potential of nanomedicine to impact oral delivery of active therapeutics (Cui, et al., 2009, Sonaje, et al., 2010).

Another important cellular barrier encountered by some small molecule therapies is that of the blood-brain interface, which forces more invasive means of local delivery, including direct injection or implantation of the naked therapeutic agent or different scaffolds containing said therapeutic into the brain, e.g., by intracerebral or intraventricular, administration (Menei, et al., 2000, Nakaji-Hirabayashi, et al., 2009, Emerich, et al., 2010). In this regard, prolonged circulation and stability rendered by nanocarriers can enhance the chances of drug diffusion into the brain parenchyma. As discussed in section 2.2.3, carriers targeted to particular transporters of the blood-brain barrier can also improve entry into the central nervous system. Examples illustrating this are those that capitalize on targeting the transferrin receptor, which provides transendothelial transport by a clathirn-mediated mechanism. This has been explored for delivery of nerve growth factor for Huntingon's disease (Kordower, et al., 1994). Nanocarriers have also been used as vehicles to assist in transporting chelating agents into the brain for iron capture and removal in Alzheimer's disease, also with potential in Huntington's and Parkinson's diseases (Liu, et al., 2009).

Similar nanomedicine strategies can improve delivery of other small molecule therapies for genetic diseases, including hormones to control regulatory pathways, antibiotics, growth factors, cofactors, inhibitors or activators that act upstream or downstream of affected pathways, chaperones that favor proper protein folding, and other chemicals.

3.2 Enzyme therapy

The term enzyme therapy describes the administration of exogenous enzymes to replace their defective endogenous counterparts (enzyme replacement therapy or ERT) and can also be extended beyond the treatment of genetic diseases with impaired enzyme production, for instance in cases where administration of additional enzymes that are not encountered endogenously in the human body can help alleviate phenotypic symptoms. Classical examples of the first approach have been applied to the treatment of lysosomal storage disorders (LSDs), mostly caused by genetic defects affecting enzymes involved in lysosomal degradation of varied substrates (Burrow, et al., 2007), neuropathic phenylketonuria (PKU) as a consequence of a defect in phenylalanine hydroxylase (PAH) (Kim, et al., 2004, Harding & Blau, 2010), or prolidase deficiency affecting collagen metabolism (Colonna, et al., 2008b), among many others. The second approach is illustrated in examples pertaining, for instance, administration of alginate lyase to degrade components of infectious biofilms in CF (Lamppa, et al., 2011) or delivery of uricase for gout treatment (Sherman, et al., 2008).

However, effective delivery of enzymes often suffers from the impediments stated above for small molecule therapies, with the added disadvantages that arise from using proteins as therapeutic agents: susceptibility to proteases, high potential for immunogenicity, and even more reduced penetration within tissues and cells in the body. Hence, these therapeutic strategies represent good targets for improvement by nanomedicine approaches.

For instance, several lysosomal ERTs are clinically available, providing a marked phenotypic improvement. However, in many cases, production of antibodies against the administered recombinant enzymes hinders the efficacy of this treatment over time, which represents a major obstacle for treatment of these chronic conditions (Ponder, 2008, Linthorst, et al., 2004). This could be ameliorated by encapsulating or coupling said enzymes within immuno-evasive carriers or polymers, such as those described in section 2.2.1. Some preliminary attempts in this direction include the case of delivery of PEG-modified dextranase, which achieved prolonged activity by bypassing immunorecognition in a mouse model of LSD mimicked by lysosomal accumulation of dextran (Mumtaz & Bachhawat, 1994). Similar strategies of PEGylation have been useful for delivery of uricase for gout

treatment in hyperuricemia (Sherman, et al., 2008), non-mammalian phenylalanine ammonia lyase for treatment of PKU (Ikeda, et al., 2005), or alginate lyase delivery targeted to CF infections (Lamppa, et al., 2011).

In addition to protecting from inactivation and immunogenic responses, by masking the enzymes from the immune and clearance systems, it is expected that these strategies involving incorporation of PEG in the formulation will also provide prolonged circulation, hence enhancing the chances to reach tissues and cell targets in the body, a capacity otherwise considerably restricted for large molecules such as these enzymes. Encapsulation within or coupling to liposomes and polymer carriers, as well as the incorporation of active targeting moieties, may help these aspects. Some examples of these applications were explored in the late 70's, such as in the case of enhanced delivery of glucocerebrosidase deficient in Gaucher disease upon encapsulation within liposomes (Dale, et al., 1979), or liposomes coupled to aggregated immunoglobulins or apolipoproteins for enhanced enzyme targeting to polymorphonucleocytes for treatment of Batten disease (Weissmann, et al., 1975, Steger & Desnick, 1977). Yet, not many subsequent works have risen from these seminal studies, adapting the rapid evolving arena of nanomedicine to formulations for treatment of genetic conditions, and both fields have developed independently. A recent attempt to bridge this gap studied enhancement of delivery of acid sphingomyelinase or α-galactosidase (deficient in types A-B Niemann-Pick disease or Fabry disease, respectively) to peripheral organs and the brain, which was achieved by coupling said enzymes to polymer nanocarriers targeted to intercellular adhesion molecule-1 (ICAM-1), a molecule present in multiple tissues and cell types in the body, whose expression is highly upregulated in many pathological conditions (Garnacho, et al., 2008, Muro, 2010, Hsu, et al., 2011).

This type of strategy can not only assists with targeting of enzymes through the body, but it can also provide access to intracellular compartments where their action is required, improving efficacy. This can be achieved by either exploiting targeting to receptors involved in endocytic transport and/or carriers capable of transporting materials across the plasma membrane or the membranous envelop of intracellular compartments (described in section 2.2.3). This is the case for prolidase delivery by chitosan nanoparticles, which enhance absorptive endocytosis of the enzyme into cells, with subsequent pH-triggered release to the cytosol (Colonna, et al., 2008a), or enhanced delivery of acid sphingomyelinase or α-galactosidase to lysosomal compartments by ICAM-1-targeted nanocarriers (Muro, et al., 2006b, Garnacho, et al., 2008, Hsu, et al., 2011). In the latter example, ICAM-1 targeting provides a means for switching the entry pathway of lysosomal enzymes within cells from clathrin-mediated endocytosis, typically induced by binding of mannose or mannose-6-phosphate residues present on the naked lysosomal enzymes to the corresponding cell receptors, to cell adhesion molecule (CAM)-mediated endocytosis induced by ICAM-1 targeting. This provides an advantage for those cases when the pathology itself tends to alter the expression or function of cell receptors, such as the case of the mannose-6-phosphate receptor in some LSDs (Dhami & Schuchman, 2004, Cardone, et al., 2008). Finally, transport across cellular barriers, greatly impaired for many enzyme therapies, could be improved by formulating said enzymes as their nanocarrier counterparts, as those described in section 2.2.3, enabling these treatment strategies to reach the central nervous system or to be converted into oral delivery modalities.

3.3 Gene therapy

In a broad definition, gene therapy encompasses the modulation of the expression of genes affected in genetic conditions, which can be achieved at the level of providing codifying

gene sequences that can enable the transcription and translation of functional proteins otherwise affected by these defects, or other regulatory sequences that can up-regulate or down-regulate said expression at any stage during transcription or translation. This is inclusive of, but not limited to, delivery of cDNA to replace the codifying sequence of the affected gene, or delivery of oligonucleotides for correction of mRNA transcripts by mRNA insertion/deletion, small interference RNA (siRNA) for silencing, etc.

This strategy capitalizes on viral vectors, given that many viruses can actively bind to cell surface receptors, enter cells by endocytosis, and gain access to the cytosol and the nucleus, in certain cases, by escaping the endo-lysosomal vesicles in which they are contained (Campbell & Hope, 2005). These viruses have evolved mechanisms capable of "sensing" the lowering pH within endosomes and lysosomes, e.g., by protonation of amphiphilic molecules, which can then destabilize and porate the endo-lysosomal membrane (Campbell & Hope, 2005). Delivery of said nucleic acid-based therapeutics has been shown to be markedly effective when using viral vectors, which is attributable to their innate ability to effectively deliver double-stranded or single-stranded DNA or RNA molecules within cells.

Most strategies attempted include somatic gene transfer in animal models using adenovirus, adeno-associated virus, herpesvirus and, more recently, retrovirus and lentivirus vectors capable of integrating the exogenous gene sequences into the host genome for prolonged expression (Wilson, 2004, Sands & Davidson, 2006). Other strategies consist of transforming cells *ex vivo* by viral vectors to express functional proteins, with subsequent local implantation of the transformed cells in the body (Ohashi, et al., 2000, Karolewski & Wolfe, 2006). However, while these strategies are highly promising, their current translational application is still limited due to cargo size limitations and mainly safety concerns that involve immunogenicity and potential for random insertions in the host genome.

In this regard, nanomedicine offers an opportunity to develop complementary approaches to traditional gene therapy. For instance, PEGylation strategies can improve the masking from the immune system, such as the case of PEGylated DNA/nanoparticles shown to provide effective intraocular transfection for retinitis pigmentosa while avoiding immune recognition (Cai, et al., 2008), or PEGylation of adenoviruses to confer protection against neutralizing antibodies (O'Riordan, et al., 1999), which may enable subsequent administrations. Similarly, encapsulation of the viral vectors themselves within polymer materials can also minimize immunogenicity, illustrated by successful gene transduction using adenoviruses encapsulated into PLGA microspheres (Turner, et al., 2007).

Encapsulation or complexation of nucleic acid material within liposomes or polymer carriers (lipoplexes or polyplexes) is also being optimized in order to avoid utilization of protein-rich viral capsids (arguably highly immunogenic) and viral genetic elements. Properties such as immune evasion, improved penetration through viscous fluids, targeting to particular cellular markers, and membrane permeabilization (those of the plasmalemma or intracellular compartments), discussed in section 2.2, are being built in these systems, to enable them to achieve delivery of nucleic acid materials. For instance, addition of albumin to polyplexes improves penetration through the sputum and provides transfection in CF (Di Gioia, et al., 2008). Bioadhesive and pH-responsive properties of chitosan nanoparticles, along with its lack of toxicity, can also benefit delivery of gene therapies (Agnihotri, et al., 2004), including oral gene delivery applications such that of coagulation factor VIII in a hemophilia mouse model (Dhadwar, et al., 2010) or glucagon-liked peptide 1 (GLP-1) to reduce blood glucose in diabetes (Jean, et al., 2011).

As discussed for other therapeutic applications, these nanomedicine strategies can be targeted to particular receptors encountered in cells where transfection is needed. As an example, this has been shown in the case of lipoplexes encoding for low-density lipoprotein receptor (LDLR) and targeted to the tranferrin receptor to provide expression of LDLR for treatment of familial hypercholesterolemia (Shichiri, et al., 2003). The same targeting strategy has been used for expression of glial-derived neurotrophic growth factor (GDNF) to reduce neurotoxicity in Parkinson's disease (Zhang, et al., 2006).

Coupling to cell penetrating peptides and/or carrier scaffolds amenable for escaping from endocytic compartments (discussed in section 2.2.3), reminiscent of viral escape from these vesicles, improves the efficacy of transfection. Examples illustrating these strategies are those of facilitated transfection by PEGylated PEI encasing oligonucleotides for Duschenne muscular dystrophy, hemophilia, and CF (Lee, et al., 2004, Carrabino, et al., 2005, Dif, et al., 2006, Sirsi, et al., 2008), lipoplexes coupled to arginine-glycine-aspartic acid (RGD) peptides that can bind to integrins (Scott, et al., 2001), or direct coupling of DNA to cell penetrating peptides, such as in the case of gene therapy for Fabry disease (Lavigne, et al., 2008).

3.4 Cell therapy

Cell therapy has been largely utilized as a therapeutic generating system, where cells from healthy subjects or cells from the patient properly modified are implanted in the body to provide sustained production of a molecule of interest that is innately defective in the diseased patient. Hence, the cell itself can be considered the vehicle of delivery and sustained effects can be achieved. This is beneficial in the case of certain proteins, hormones, and small molecules (such as neurotrophic factors) that display short circulatory longevity and are easily subjected to proteases, shortening their therapeutic effects.

By directly producing these molecules at the required site, cell therapy provides a means to bypass secondary strategies to stabilize said molecules or the necessity for enabling crossing of physiological barriers. This is the case for implantation of alginate microparticles carrying recombinant fibroblasts for sustained systemic release of α-glucuronidase for mucopolysaccharidosis VII (Ross, et al., 2000a). Local implantation of the encapsulated cells into the brain lateral ventricles circumvents the blood-brain barrier and results in considerable delivery of the secreted enzyme (Ross, et al., 2000b). Similarly, implantation of encapsulated, genetically modified fibroblasts capable of producing ciliary neurotrophic factor (CNTF), a neuroprotective agent, in the brain or within vitreous humor of the eye have been explored in the context of treatment for Huntington's disease and retinitis pigmentosa (Emerich, et al., 1996, Tao, et al., 2002, Bloch, et al., 2004).

In most of these applications, encapsulation of cells to be implanted within polymer matrices is advantageous, if not required, in order to create a protective physical barrier to the immune system while permitting sustained release of the therapeutic by the implanted cells. Such scaffolds can avoid unwanted cell escape and neoplastic growth, yet allow oxygen and nutrients to access the matrix to maintain cell viability, while permitting therapeutics to be secreted to the surrounding environment. In this regard, nanotechnology has permitted nano-scale manipulation of these materials to achieve controlled diffusion, limiting immune response while supporting sustained release of the therapeutic molecules. An example of this strategy capitalizes on the use of alginate poly(L-lysine) hydrogels, an immune elusive and non-toxic polymer, (Koch, et al., 2003) with a nanoporous architecture, suitable to allow minimal yet sufficient nutrient exchange (Desai, et al., 2004).

Reduced access to elements of the immune system may also provide an avenue for use of allografts or xenografts, as in the case of long term implantation into rats of bovine islet cells encapsulated within alginate poly(L-lysine) microcapsules (Lanza, et al., 1999). Similarly, this property can be exploited to achieve more sustained therapeutic effects, such as those obtained in the case of genetically modified endothelial cells in treatment of hemophilia A, effective for ~150 days (Lin, et al., 2002), modified myoblasts to secrete erythropoietin, hence reducing the need of constant blood transfusions and hormone injections for treatment of the thalassemias (Dalle, et al., 1999), or encapsulated fibroblasts that release GDNF for Parkinson's disease, which was effective for up to 2 years (Zurn, et al., 2001).

4. Conclusion

During the last 2-3 decades, the development of nanotechnologies for medical applications has provided important new avenues to improve diagnosis and treatment of human maladies. Said nanomedicines display a considerable degree of control of parameters such as increased solubility of various cargoes, evasion from the immune system, controlled circulation, improved stability, enhanced biodistribution in the body, modulatable transport into and/or across cells, and controlled release at the final destination. These approaches can be applied to the development of sensors and imaging agents with improved detection and diagnostic sensitivity, and also delivery of therapeutic strategies pertaining to small molecule, enzyme, gene, and cell therapies. Several nanoconstructs with varied chemical nature, architectural design, and functional properties have been successfully translated into the clinics, mainly for applications other than treatment of genetic conditions. Although, their application in this field has been only modestly explored, these systems rather represent general platforms, offering a unique opportunity to develop alternative and complementary therapeutic interventions applicable to the treatment of genetic diseases.

5. Acknowledgments

6. References

Agnihotri, S. A., Mallikarjuna, N. N. & Aminabhavi, T. M. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J Control Release*, Vol.100, No.1, (Nov 5), pp. 5-28, 0168-3659 (Print), 0168-3659 (Linking)

Bailey, V. J., Puleo, C. M., Ho, Y. P., Yeh, H. C. & Wang, T. H. (2009). Quantum dots in molecular detection of disease. *Conf Proc IEEE Eng Med Biol Soc*, Vol.2009, pp. 4089-4092, 1557-170X (Print), 1557-170X (Linking)

Bareford, L. M. & Swaan, P. W. (2007). Endocytic mechanisms for targeted drug delivery. *Adv Drug Deliv Rev*, Vol.59, No.8, (Aug 10), pp. 748-758, 0169-409X (Print), 0169-409X (Linking)

Bloch, J., Bachoud-Levi, A. C., Deglon, N., Lefaucheur, J. P., Winkel, L., Palfi, S., Nguyen, J. P., Bourdet, C., Gaura, V., Remy, P., Brugieres, P., Boisse, M. F., Baudic, S., Cesaro, P., Hantraye, P., Aebischer, P. & Peschanski, M. (2004). Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: results of a phase I study. *Hum Gene Ther*, Vol.15, No.10, (Oct), pp. 968-975, 1043-0342 (Print), 1043-0342 (Linking)

Burrow, T. A., Hopkin, R. J., Leslie, N. D., Tinkle, B. T. & Grabowski, G. A. (2007). Enzyme reconstitution/replacement therapy for lysosomal storage diseases. *Curr Opin Pediatr*, Vol.19, No.6, (Dec), pp. 628-635, 1040-8703 (Print), 1040-8703 (Linking)

Cai, H., Zhu, N., Jiang, Y., He, P. & Fang, Y. (2003). Cu@Au alloy nanoparticle as oligonucleotides labels for electrochemical stripping detection of DNA hybridization. *Biosens Bioelectron*, Vol.18, No.11, (Oct 1), pp. 1311-1319, 0956-5663 (Print), 0956-5663 (Linking)

Cai, X., Conley, S. & Naash, M. (2008). Nanoparticle applications in ocular gene therapy. *Vision Res*, Vol.48, No.3, (Feb), pp. 319-324, 0042-6989 (Print), 0042-6989 (Linking)

Campbell, E. M. & Hope, T. J. (2005). Gene therapy progress and prospects: viral trafficking during infection. *Gene Ther*, Vol.12, No.18, (Sep), pp. 1353-1359,

Cardone, M., Porto, C., Tarallo, A., Vicinanza, M., Rossi, B., Polishchuk, E., Donaudy, F., Andria, G., De Matteis, M. A. & Parenti, G. (2008). Abnormal mannose-6-phosphate receptor trafficking impairs recombinant alpha-glucosidase uptake in Pompe disease fibroblasts. *Pathogenetics*, Vol.1, No.1, pp. 6, 1755-8417 (Electronic)

Carrabino, S., Di Gioia, S., Copreni, E. & Conese, M. (2005). Serum albumin enhances polyethylenimine-mediated gene delivery to human respiratory epithelial cells. *J Gene Med*, Vol.7, No.12, (Dec), pp. 1555-1564, 1099-498X (Print), 1099-498X (Linking)

Cartiera, M. S., Ferreira, E. C., Caputo, C., Egan, M. E., Caplan, M. J. & Saltzman, W. M. (2010). Partial correction of cystic fibrosis defects with PLGA nanoparticles encapsulating curcumin. *Mol Pharm*, Vol.7, No.1, (Feb 1), pp. 86-93, 1543-8392 (Electronic), 1543-8384 (Linking)

Chen, R. J., Bangsaruntip, S., Drouvalakis, K. A., Kam, N. W., Shim, M., Li, Y., Kim, W., Utz, P. J. & Dai, H. (2003). Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proc Natl Acad Sci U S A*, Vol.100, No.9, (Apr 29), pp. 4984-4989, 0027-8424 (Print), 0027-8424 (Linking)

Cheung, W., Pontoriero, F., Taratula, O., Chen, A. M. & He, H. (2010). DNA and carbon nanotubes as medicine. *Adv Drug Deliv Rev*, Vol.62, No.6, (Apr 30), pp. 633-649, 1872-8294 (Electronic), 0169-409X (Linking)

Choi, H. S. & Frangioni, J. V. (2010). Nanoparticles for biomedical imaging: fundamentals of clinical translation. *Mol Imaging*, Vol.9, No.6, (Dec), pp. 291-310, 1536-0121 (Electronic), 1535-3508 (Linking)

Choi, S. H., Lee, S. H. & Park, T. G. (2006). Temperature-Sensitive Pluronic/Poly(ethylenimine) Nanocapsules for Thermally Triggered Disruption of Intracellular Endosomal Compartment. *Biomacromolecules*, Vol.7, No.6, (Jun), pp. 1864-1870,

Chougule, M. B., Padhi, B. K. & Misra, A. (2006). Nano-liposomal dry powder inhaler of Amiloride Hydrochloride. *J Nanosci Nanotechnol*, Vol.6, No.9-10, (Sep-Oct), pp. 3001-3009, 1533-4880 (Print), 1533-4880 (Linking)

Colonna, C., Conti, B., Perugini, P., Pavanetto, F., Modena, T., Dorati, R., Iadarola, P. & Genta, I. (2008a). Ex vivo evaluation of prolidase loaded chitosan nanoparticles for the enzyme replacement therapy. *Eur J Pharm Biopharm*, Vol.70, No.1, (Sep), pp. 58-65, 0939-6411 (Print), 0939-6411 (Linking)

Colonna, C., Conti, B., Perugini, P., Pavanetto, F., Modena, T., Dorati, R., Iadarola, P. & Genta, I. (2008b). Site-directed PEGylation as successful approach to improve the enzyme replacement in the case of prolidase. *Int J Pharm*, Vol.358, No.1-2, (Jun 24), pp. 230-237, 0378-5173 (Print), 0378-5173 (Linking)

Corstjens, P. L., Li, S., Zuiderwijk, M., Kardos, K., Abrams, W. R., Niedbala, R. S. & Tanke, H. J. (2005). Infrared up-converting phosphors for bioassays. *IEE Proc Nanobiotechnol*, Vol.152, No.2, (Apr), pp. 64-72, 1478-1581 (Print), 1478-1581 (Linking)

Cui, F., Qian, F., Zhao, Z., Yin, L., Tang, C. & Yin, C. (2009). Preparation, characterization, and oral delivery of insulin loaded carboxylated chitosan grafted poly(methyl methacrylate) nanoparticles. *Biomacromolecules*, Vol.10, No.5, (May 11), pp. 1253-1258, 1526-4602 (Electronic). 1525-7797 (Linking)

Dale, G. L., Kuhl, W. & Beutler, E. (1979). Incorporation of glucocerebrosidase into Gaucher's disease monocytes in vitro. *Proc Natl Acad Sci U S A*, Vol.76, No.1, (Jan), pp. 473-475, 0027-8424 (Print), 0027-8424 (Linking)

Dalle, B., Payen, E., Regulier, E., Deglon, N., Rouyer-Fessard, P., Beuzard, Y. & Aebischer, P. (1999). Improvement of mouse beta-thalassemia upon erythropoietin delivery by encapsulated myoblasts. *Gene Ther*, Vol.6, No.2, (Feb), pp. 157-161, 0969-7128 (Print), 0969-7128 (Linking)

Dejana, E. (2004). Endothelial cell-cell junctions: happy together. *Nat Rev Mol Cell Biol*, Vol.5, No.4, (Apr), pp. 261-270,

Desai, T. A., West, T., Cohen, M., Boiarski, T. & Rampersaud, A. (2004). Nanoporous microsystems for islet cell replacement. *Adv Drug Deliv Rev*, Vol.56, No.11, (Sep 22), pp. 1661-1673, 0169-409X (Print), 0169-409X (Linking)

Dhadwar, S. S., Kiernan, J., Wen, J. & Hortelano, G. (2010). Repeated oral administration of chitosan/DNA nanoparticles delivers functional FVIII with the absence of antibodies in hemophilia A mice. *J Thromb Haemost*, Vol.8, No.12, (Dec), pp. 2743-2750, 1538-7836 (Electronic), 1538-7836 (Linking)

Dhami, R. & Schuchman, E. H. (2004). Mannose 6-phosphate receptor-mediated uptake is defective in acid sphingomyelinase-deficient macrophages: implications for Niemann-Pick disease enzyme replacement therapy. *J Biol Chem*, Vol.279, No.2, (Jan 9), pp. 1526-1532,

Di Gioia, S., Rejman, J., Carrabino, S., De Fino, I., Rudolph, C., Doherty, A., Hyndman, L., Di Cicco, M., Copreni, E., Bragonzi, A., Colombo, C., Boyd, A. C. & Conese, M. (2008). Role of biophysical parameters on ex vivo and in vivo gene transfer to the airway epithelium by polyethylenimine/albumin complexes. *Biomacromolecules*, Vol.9, No.3, (Mar), pp. 859-866, 1526-4602 (Electronic), 1525-7797 (Linking)

Dif, F., Djediat, C., Alegria, O., Demeneix, B. & Levi, G. (2006). Transfection of multiple pulmonary cell types following intravenous injection of PEI-DNA in normal and CFTR mutant mice. *J Gene Med*, Vol.8, No.1, (Jan), pp. 82-89, 1099-498X (Print), 1099-498X (Linking)

Dincer, S., Turk, M. & Piskin, E. (2005). Intelligent polymers as nonviral vectors. *Gene Ther*, Vol.12 Suppl 1, (Oct), pp. S139-145,

Discher, D. E. & Eisenberg, A. (2002). Polymer vesicles. *Science*, Vol.297, No.5583, (Aug 9), pp. 967-973,

Duncan, R. (2003). The dawning era of polymer therapeutics. *Nat Rev Drug Discov*, Vol.2, No.5, (May), pp. 347-360,

El-Sayed, M. E., Hoffman, A. S. & Stayton, P. S. (2005). Smart polymeric carriers for enhanced intracellular delivery of therapeutic macromolecules. *Expert Opin Biol Ther*, Vol.5, No.1, (Jan), pp. 23-32,

Emerich, D. F., Lindner, M. D., Winn, S. R., Chen, E. Y., Frydel, B. R. & Kordower, J. H. (1996). Implants of encapsulated human CNTF-producing fibroblasts prevent behavioral deficits and striatal degeneration in a rodent model of Huntington's disease. *J Neurosci*, Vol.16, No.16, (Aug 15), pp. 5168-5181, 0270-6474 (Print), 0270-6474 (Linking)

Emerich, D. F., Mooney, D. J., Storrie, H., Babu, R. S. & Kordower, J. H. (2010). Injectable hydrogels providing sustained delivery of vascular endothelial growth factor are neuroprotective in a rat model of Huntington's disease. *Neurotox Res*, Vol.17, No.1, (Jan), pp. 66-74, 1476-3524 (Electronic), 1029-8428 (Linking)

Garnacho, C., Dhami, R., Simone, E., Dziubla, T., Leferovich, J., Schuchman, E. H., Muzykantov, V. & Muro, S. (2008). Delivery of acid sphingomyelinase in normal and niemann-pick disease mice using intercellular adhesion molecule-1-targeted polymer nanocarriers. *J Pharmacol Exp Ther*, Vol.325, No.2, (May), pp. 400-408, 1521-0103 (Electronic), 0022-3565 (Linking)

Halwani, M., Yebio, B., Suntres, Z. E., Alipour, M., Azghani, A. O. & Omri, A. (2008). Co-encapsulation of gallium with gentamicin in liposomes enhances antimicrobial activity of gentamicin against Pseudomonas aeruginosa. *J Antimicrob Chemother*, Vol.62, No.6, (Dec), pp. 1291-1297, 1460-2091 (Electronic), 0305-7453 (Linking)

Harding, C. O. & Blau, N. (2010). Advances and challenges in phenylketonuria. *J Inherit Metab Dis*, Vol.33, No.6, (Dec), pp. 645-648, 1573-2665 (Electronic), 0141-8955 (Linking)

Hilgenbrink, A. R. & Low, P. S. (2005). Folate receptor-mediated drug targeting: from therapeutics to diagnostics. *J Pharm Sci*, Vol.94, No.10, (Oct), pp. 2135-2146, 0022-3549 (Print)

Hsu, J., Serrano, D., Bhowmick, T., Kumar, K., Shen, Y., Kuo, Y. C., Garnacho, C. & Muro, S. (2011). Enhanced endothelial delivery and biochemical effects of alpha-galactosidase by ICAM-1-targeted nanocarriers for Fabry disease. *J Control Release*, Vol.149, No.3, (Feb 10), pp. 323-331, 1873-4995 (Electronic), 0168-3659 (Linking)

Ikeda, K., Schiltz, E., Fujii, T., Takahashi, M., Mitsui, K., Kodera, Y., Matsushima, A., Inada, Y., Schulz, G. E. & Nishimura, H. (2005). Phenylalanine ammonia-lyase modified

with polyethylene glycol: potential therapeutic agent for phenylketonuria. *Amino Acids*, Vol.29, No.3, (Nov), pp. 283-287, 0939-4451 (Print), 0939-4451 (Linking)

Jean, M., Alameh, M., Buschmann, M. D. & Merzouki, A. (2011). Effective and safe gene-based delivery of GLP-1 using chitosan/plasmid-DNA therapeutic nanocomplexes in an animal model of type 2 diabetes. *Gene Ther*, (Mar 17), pp. 1476-5462 (Electronic), 0969-7128 (Linking)

Kakudo, T., Chaki, S., Futaki, S., Nakase, I., Akaji, K., Kawakami, T., Maruyama, K., Kamiya, H. & Harashima, H. (2004). Transferrin-modified liposomes equipped with a pH-sensitive fusogenic peptide: an artificial viral-like delivery system. *Biochemistry*, Vol.43, No.19, (May 18), pp. 5618-5628,

Kamaly, N. & Miller, A. D. (2010). Paramagnetic liposome nanoparticles for cellular and tumour imaging. *Int J Mol Sci*, Vol.11, No.4, pp. 1759-1776, 1422-0067 (Electronic), 1422-0067 (Linking)

Karolewski, B. A. & Wolfe, J. H. (2006). Genetic correction of the fetal brain increases the lifespan of mice with the severe multisystemic disease mucopolysaccharidosis type VII. *Mol Ther*, Vol.14, No.1, (Jul), pp. 14-24, 1525-0016 (Print)

Kim, W., Erlandsen, H., Surendran, S., Stevens, R. C., Gamez, A., Michols-Matalon, K., Tyring, S. K. & Matalon, R. (2004). Trends in enzyme therapy for phenylketonuria. *Mol Ther*, Vol.10, No.2, (Aug), pp. 220-224, 1525-0016 (Print), 1525-0016 (Linking)

Kitchens, K. M., El-Sayed, M. E. & Ghandehari, H. (2005). Transepithelial and endothelial transport of poly (amidoamine) dendrimers. *Adv Drug Deliv Rev*, Vol.57, No.15, (Dec 14), pp. 2163-2176, 0169-409X (Print)

Koch, S., Schwinger, C., Kressler, J., Heinzen, C. & Rainov, N. G. (2003). Alginate encapsulation of genetically engineered mammalian cells: comparison of production devices, methods and microcapsule characteristics. *J Microencapsul*, Vol.20, No.3, (May-Jun), pp. 303-316, 0265-2048 (Print), 0265-2048 (Linking)

Kordower, J. H., Charles, V., Bayer, R., Bartus, R. T., Putney, S., Walus, L. R. & Friden, P. M. (1994). Intravenous administration of a transferrin receptor antibody-nerve growth factor conjugate prevents the degeneration of cholinergic striatal neurons in a model of Huntington disease. *Proc Natl Acad Sci U S A*, Vol.91, No.19, (Sep 13), pp. 9077-9080, 0027-8424 (Print), 0027-8424 (Linking)

Lamppa, J. W., Ackerman, M. E., Lai, J. I., Scanlon, T. C. & Griswold, K. E. (2011). Genetically engineered alginate lyase-PEG conjugates exhibit enhanced catalytic function and reduced immunoreactivity. *PLoS One*, Vol.6, No.2, pp. e17042, 1932-6203 (Electronic), 1932-6203 (Linking)

Langer, R. (1998). Drug delivery and targeting. *Nature*, Vol.392, No.6679 Suppl, (Apr 30), pp. 5-10,

Lanza, R. P., Jackson, R., Sullivan, A., Ringeling, J., McGrath, C., Kuhtreiber, W. & Chick, W. L. (1999). Xenotransplantation of cells using biodegradable microcapsules. *Transplantation*, Vol.67, No.8, (Apr 27), pp. 1105-1111, 0041-1337 (Print), 0041-1337 (Linking)

Lavigne, M. D., Yates, L., Coxhead, P. & Gorecki, D. C. (2008). Nuclear-targeted chimeric vector enhancing nonviral gene transfer into skeletal muscle of Fabry mice in vivo.

FASEB J, Vol.22, No.6, (Jun), pp. 2097-2107, 1530-6860 (Electronic), 0892-6638 (Linking)

Lee, J. S., Lee, M. & Kim, S. W. (2004). A new potent hFIX plasmid for hemophilia B gene therapy. *Pharm Res*, Vol.21, No.7, (Jul), pp. 1229-1232, 0724-8741 (Print), 0724-8741 (Linking)

Lin, Y., Chang, L., Solovey, A., Healey, J. F., Lollar, P. & Hebbel, R. P. (2002). Use of blood outgrowth endothelial cells for gene therapy for hemophilia A. *Blood*, Vol.99, No.2, (Jan 15), pp. 457-462, 0006-4971 (Print), 0006-4971 (Linking)

Linthorst, G. E., Hollak, C. E., Donker-Koopman, W. E., Strijland, A. & Aerts, J. M. (2004). Enzyme therapy for Fabry disease: neutralizing antibodies toward agalsidase alpha and beta. *Kidney Int*, Vol.66, No.4, (Oct), pp. 1589-1595, 0085-2538 (Print)

Liu, G., Men, P., Perry, G. & Smith, M. A. (2009). Metal chelators coupled with nanoparticles as potential therapeutic agents for Alzheimer's disease. *J Nanoneurosci*, Vol.1, No.1, (Jun 1), pp. 42-55, 1939-0653 (Electronic)

Magzoub, M., Pramanik, A. & Graslund, A. (2005). Modeling the endosomal escape of cell-penetrating peptides: transmembrane pH gradient driven translocation across phospholipid bilayers. *Biochemistry*, Vol.44, No.45, (Nov 15), pp. 14890-14897,

Menei, P., Pean, J. M., Nerriere-Daguin, V., Jollivet, C., Brachet, P. & Benoit, J. P. (2000). Intracerebral implantation of NGF-releasing biodegradable microspheres protects striatum against excitotoxic damage. *Exp Neurol*, Vol.161, No.1, (Jan), pp. 259-272, 0014-4886 (Print), 0014-4886 (Linking)

Minko, T., Pakunlu, R. I., Wang, Y., Khandare, J. J. & Saad, M. (2006). New generation of liposomal drugs for cancer. *Anticancer Agents Med Chem*, Vol.6, No.6, (Nov), pp. 537-552, 1871-5206 (Print), 1871-5206 (Linking)

Moghimi, S. M., Hunter, A. C. & Murray, J. C. (2001). Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol Rev*, Vol.53, No.2, (Jun), pp. 283-318,

Moghimi, S. M., Hunter, A. C. & Murray, J. C. (2005). Nanomedicine: current status and future prospects. *FASEB J*, Vol.19, No.3, (Mar), pp. 311-330, 1530-6860 (Electronic), 0892-6638 (Linking)

Moghimi, S. M. & Szebeni, J. (2003). Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. *Prog Lipid Res*, Vol.42, No.6, (Nov), pp. 463-478,

Mugabe, C., Azghani, A. O. & Omri, A. (2005). Liposome-mediated gentamicin delivery: development and activity against resistant strains of Pseudomonas aeruginosa isolated from cystic fibrosis patients. *J Antimicrob Chemother*, Vol.55, No.2, (Feb), pp. 269-271, 0305-7453 (Print), 0305-7453 (Linking)

Mumtaz, S. & Bachhawat, B. K. (1994). Enhanced intracellular stability and efficacy of PEG modified dextranase in the treatment of a model storage disorder. *Biochim Biophys Acta*, Vol.1199, No.2, (Mar 2), pp. 175-182,

Mundargi, R. C., Babu, V. R., Rangaswamy, V., Patel, P. & Aminabhavi, T. M. (2008). Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. *J Control Release*, Vol.125, No.3, (Feb 11), pp. 193-209, 1873-4995 (Electronic), 0168-3659 (Linking)

Muro, S. (2010). New biotechnological and nanomedicine strategies for treatment of lysosomal storage disorders. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, Vol.2, No.2, (Mar-Apr), pp. 189-204, 1939-0041 (Electronic), 1939-0041 (Linking)

Muro, S., Dziubla, T., Qiu, W., Leferovich, J., Cui, X., Berk, E. & Muzykantov, V. R. (2006a). Endothelial targeting of high-affinity multivalent polymer nanocarriers directed to intercellular adhesion molecule 1. *J Pharmacol Exp Ther*, Vol.317, No.3, (Jun), pp. 1161-1169,

Muro, S., Garnacho, C., Champion, J. A., Leferovich, J., Gajewski, C., Schuchman, E. H., Mitragotri, S. & Muzykantov, V. R. (2008). Control of endothelial targeting and intracellular delivery of therapeutic enzymes by modulating the size and shape of ICAM-1-targeted carriers. *Mol Ther*, Vol.16, No.8, (Aug), pp. 1450-1458, 1525-0024 (Electronic)

Muro, S., Koval, M. & Muzykantov, V. (2004). Endothelial endocytic pathways: gates for vascular drug delivery. *Curr Vasc Pharmacol*, Vol.2, No.3, (Jul), pp. 281-299,

Muro, S. & Muzykantov, V. R. (2005). Targeting of antioxidant and anti-thrombotic drugs to endothelial cell adhesion molecules. *Curr Pharm Des*, Vol.11, No.18, pp. 2383-2401,

Muro, S., Schuchman, E. H. & Muzykantov, V. R. (2006b). Lysosomal enzyme delivery by ICAM-1-targeted nanocarriers bypassing glycosylation- and clathrin-dependent endocytosis. *Mol Ther*, Vol.13, No.1, (Jan), pp. 135-141,

Musacchio, T. & Torchilin, V. P. (2011). Recent developments in lipid-based pharmaceutical nanocarriers. *Front Biosci*, Vol.16, pp. 1388-1412, 1093-4715 (Electronic), 1093-4715 (Linking)

Nacev, A., Beni, C., Bruno, O. & Shapiro, B. (2010). Magnetic nanoparticle transport within flowing blood and into surrounding tissue. *Nanomedicine (Lond)*, Vol.5, No.9, (Nov), pp. 1459-1466, 1748-6963 (Electronic), 1743-5889 (Linking)

Nakaji-Hirabayashi, T., Kato, K. & Iwata, H. (2009). Hyaluronic acid hydrogel loaded with genetically-engineered brain-derived neurotrophic factor as a neural cell carrier. *Biomaterials*, Vol.30, No.27, (Sep), pp. 4581-4589, 1878-5905 (Electronic), 0142-9612 (Linking)

Nnamani, P. O., Attama, A. A., Ibezim, E. C. & Adikwu, M. U. (2010). SRMS142-based solid lipid microparticles: application in oral delivery of glibenclamide to diabetic rats. *Eur J Pharm Biopharm*, Vol.76, No.1, (Sep), pp. 68-74, 1873-3441 (Electronic), 0939-6411 (Linking)

O'Brien, J. A., Holt, M., Whiteside, G., Lummis, S. C. & Hastings, M. H. (2001). Modifications to the hand-held Gene Gun: improvements for in vitro biolistic transfection of organotypic neuronal tissue. *J Neurosci Methods*, Vol.112, No.1, (Nov 15), pp. 57-64, 0165-0270 (Print), 0165-0270 (Linking)

O'Riordan, C. R., Lachapelle, A., Delgado, C., Parkes, V., Wadsworth, S. C., Smith, A. E. & Francis, G. E. (1999). PEGylation of adenovirus with retention of infectivity and protection from neutralizing antibody in vitro and in vivo. *Hum Gene Ther*, Vol.10, No.8, (May 20), pp. 1349-1358, 1043-0342 (Print), 1043-0342 (Linking)

Ohashi, T., Yokoo, T., Iizuka, S., Kobayashi, H., Sly, W. S. & Eto, Y. (2000). Reduction of lysosomal storage in murine mucopolysaccharidosis type VII by transplantation of

normal and genetically modified macrophages. *Blood*, Vol.95, No.11, (Jun 1), pp. 3631-3633, 0006-4971 (Print)

Oishi, M., Kataoka, K. & Nagasaki, Y. (2006). pH-responsive three-layered PEGylated polyplex micelle based on a lactosylated ABC triblock copolymer as a targetable and endosome-disruptive nonviral gene vector. *Bioconjug Chem*, Vol.17, No.3, (May-Jun), pp. 677-688,

Panyam, J. & Labhasetwar, V. (2003). Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv Drug Deliv Rev*, Vol.55, No.3, (Feb 24), pp. 329-347,

Panyam, J., Zhou, W. Z., Prabha, S., Sahoo, S. K. & Labhasetwar, V. (2002). Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. *FASEB J*, Vol.16, No.10, (Aug), pp. 1217-1226,

Pardridge, W. M. (2010). Biopharmaceutical drug targeting to the brain. *J Drug Target*, Vol.18, No.3, (Apr), pp. 157-167, 1029-2330 (Electronic), 1026-7158 (Linking)

Park, K., Kwon, I. C. & Park, K. (2011). Oral protein delivery: Current status and future prospect *Reactive and Functional Polymers*, Vol.71, No.3, (March 2011), pp. 280-287,

Ponder, K. P. (2008). Immune response hinders therapy for lysosomal storage diseases. *J Clin Invest*, Vol.118, No.8, (Aug), pp. 2686-2689, 0021-9738 (Print)

Ross, C. J., Bastedo, L., Maier, S. A., Sands, M. S. & Chang, P. L. (2000a). Treatment of a lysosomal storage disease, mucopolysaccharidosis VII, with microencapsulated recombinant cells. *Hum Gene Ther*, Vol.11, No.15, (Oct 10), pp. 2117-2127, 1043-0342 (Print), 1043-0342 (Linking)

Ross, C. J., Ralph, M. & Chang, P. L. (2000b). Somatic gene therapy for a neurodegenerative disease using microencapsulated recombinant cells. *Exp Neurol*, Vol.166, No.2, (Dec), pp. 276-286, 0014-4886 (Print), 0014-4886 (Linking)

Rukholm, G., Mugabe, C., Azghani, A. O. & Omri, A. (2006). Antibacterial activity of liposomal gentamicin against Pseudomonas aeruginosa: a time-kill study. *Int J Antimicrob Agents*, Vol.27, No.3, (Mar), pp. 247-252, 0924-8579 (Print), 0924-8579 (Linking)

Sahay, G., Alakhova, D. Y. & Kabanov, A. V. (2010). Endocytosis of nanomedicines. *J Control Release*, Vol.145, No.3, (Aug 3), pp. 182-195, 1873-4995 (Electronic), 0168-3659 (Linking)

Sands, M. S. & Davidson, B. L. (2006). Gene therapy for lysosomal storage diseases. *Mol Ther*, Vol.13, No.5, (May), pp. 839-849, 1525-0016 (Print)

Sawant, R. & Torchilin, V. (2010). Intracellular transduction using cell-penetrating peptides. *Mol Biosyst*, Vol.6, No.4, (Apr), pp. 628-640, 1742-2051 (Electronic), 1742-2051 (Linking)

Scott, E. S., Wiseman, J. W., Evans, M. J. & Colledge, W. H. (2001). Enhanced gene delivery to human airway epithelial cells using an integrin-targeting lipoplex. *J Gene Med*, Vol.3, No.2, (Mar-Apr), pp. 125-134, 1099-498X (Print), 1099-498X (Linking)

Serra, L., Domenech, J. & Peppas, N. A. (2009). Engineering design and molecular dynamics of mucoadhesive drug delivery systems as targeting agents. *Eur J Pharm Biopharm*, Vol.71, No.3, (Mar), pp. 519-528, 1873-3441 (Electronic), 0939-6411 (Linking)

Sherman, M. R., Saifer, M. G. & Perez-Ruiz, F. (2008). PEG-uricase in the management of treatment-resistant gout and hyperuricemia. *Adv Drug Deliv Rev*, Vol.60, No.1, (Jan 3), pp. 59-68, 0169-409X (Print), 0169-409X (Linking)

Shichiri, M., Tanaka, A. & Hirata, Y. (2003). Intravenous gene therapy for familial hypercholesterolemia using ligand-facilitated transfer of a liposome:LDL receptor gene complex. *Gene Ther*, Vol.10, No.9, (May), pp. 827-831, 0969-7128 (Print), 0969-7128 (Linking)

Shubayev, V. I., Pisanic, T. R., 2nd & Jin, S. (2009). Magnetic nanoparticles for theragnostics. *Adv Drug Deliv Rev*, Vol.61, No.6, (Jun 21), pp. 467-477, 1872-8294 (Electronic), 0169-409X (Linking)

Sirsi, S. R., Schray, R. C., Guan, X., Lykens, N. M., Williams, J. H., Erney, M. L. & Lutz, G. J. (2008). Functionalized PEG-PEI copolymers complexed to exon-skipping oligonucleotides improve dystrophin expression in mdx mice. *Hum Gene Ther*, Vol.19, No.8, (Aug), pp. 795-806, 1557-7422 (Electronic), 1043-0342 (Linking)

Sonaje, K., Chen, Y. J., Chen, H. L., Wey, S. P., Juang, J. H., Nguyen, H. N., Hsu, C. W., Lin, K. J. & Sung, H. W. (2010). Enteric-coated capsules filled with freeze-dried chitosan/poly(gamma-glutamic acid) nanoparticles for oral insulin delivery. *Biomaterials*, Vol.31, No.12, (Apr), pp. 3384-3394, 1878-5905 (Electronic), 0142-9612 (Linking)

Sonawane, N. D., Szoka, F. C., Jr. & Verkman, A. S. (2003). Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. *J Biol Chem*, Vol.278, No.45, (Nov 7), pp. 44826-44831, 0021-9258 (Print), 0021-9258 (Linking)

Stachelek, S. J., Finley, M. J., Alferiev, I. S., Wang, F., Tsai, R. K., Eckells, E. C., Tomczyk, N., Connolly, J. M., Discher, D. E., Eckmann, D. M. & Levy, R. J. (2011). The effect of CD47 modified polymer surfaces on inflammatory cell attachment and activation. *Biomaterials*, Vol.32, No.19, (Jul), pp. 4317-4326, 1878-5905 (Electronic), 0142-9612 (Linking)

Stayton, P. S., El-Sayed, M. E., Murthy, N., Bulmus, V., Lackey, C., Cheung, C. & Hoffman, A. S. (2005). 'Smart' delivery systems for biomolecular therapeutics. *Orthod Craniofac Res*, Vol.8, No.3, (Aug), pp. 219-225,

Steger, L. D. & Desnick, R. J. (1977). Enzyme therapy. VI: Comparative in vivo fates and effects on lysosomal integrity of enzyme entrapped in negatively and positively charged liposomes. *Biochim Biophys Acta*, Vol.464, No.3, (Feb 4), pp. 530-546, 0006-3002 (Print)

Suk, J. S., Suh, J., Choy, K., Lai, S. K., Fu, J. & Hanes, J. (2006). Gene delivery to differentiated neurotypic cells with RGD and HIV Tat peptide functionalized polymeric nanoparticles. *Biomaterials*, (Oct), pp. 5143-5150.

Tao, W., Wen, R., Goddard, M. B., Sherman, S. D., O'Rourke, P. J., Stabila, P. F., Bell, W. J., Dean, B. J., Kauper, K. A., Budz, V. A., Tsiaras, W. G., Acland, G. M., Pearce-Kelling, S., Laties, A. M. & Aguirre, G. D. (2002). Encapsulated cell-based delivery of CNTF reduces photoreceptor degeneration in animal models of retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, Vol.43, No.10, (Oct), pp. 3292-3298, 0146-0404 (Print), 0146-0404 (Linking)

Tokarev, I. & Minko, S. (2010). Stimuli-responsive porous hydrogels at interfaces for molecular filtration, separation, controlled release, and gating in capsules and membranes. *Adv Mater*, Vol.22, No.31, (Aug 17), pp. 3446-3462, 1521-4095 (Electronic), 0935-9648 (Linking)

Torchilin, V. P. (2006). Multifunctional nanocarriers. *Adv Drug Deliv Rev*, Vol.58, No.14, (Dec 1), pp. 1532-1555, 0169-409X (Print)

Trollet, C., Scherman, D. & Bigey, P. (2008). Delivery of DNA into muscle for treating systemic diseases: advantages and challenges. *Methods Mol Biol*, Vol.423, pp. 199-214, 1064-3745 (Print), 1064-3745 (Linking)

Turner, P., Petch, A. & Al-Rubeai, M. (2007). Encapsulation of viral vectors for gene therapy applications. *Biotechnol Prog*, Vol.23, No.2, (Mar-Apr), pp. 423-429, 8756-7938 (Print), 1520-6033 (Linking)

Vij, N., Min, T., Marasigan, R., Belcher, C. N., Mazur, S., Ding, H., Yong, K. T. & Roy, I. (2010). Development of PEGylated PLGA nanoparticle for controlled and sustained drug delivery in cystic fibrosis. *J Nanobiotechnology*, Vol.8, pp. 22, 1477-3155 (Electronic), 1477-3155 (Linking)

Weissmann, G., Bloomgarden, D., Kaplan, R., Cohen, C., Hoffstein, S., Collins, T., Gotlieb, A. & Nagle, D. (1975). A general method for the introduction of enzymes, by means of immunoglobulin-coated liposomes, into lysosomes of deficient cells. *Proc Natl Acad Sci U S A*, Vol.72, No.1, (Jan), pp. 88-92, 0027-8424 (Print)

Wilson, J. M. (2004). Adeno-associated virus and lentivirus pseudotypes for lung-directed gene therapy. *Proc Am Thorac Soc*, Vol.1, No.4, pp. 309-314, 1546-3222 (Print), 1546-3222 (Linking)

Wong, T. W. (2010). Design of oral insulin delivery systems. *J Drug Target*, Vol.18, No.2, (Feb), pp. 79-92, 1029-2330 (Electronic), 1026-7158 (Linking)

Yatuv, R., Robinson, M., Dayan-Tarshish, I. & Baru, M. (2010). The use of PEGylated liposomes in the development of drug delivery applications for the treatment of hemophilia. *Int J Nanomedicine*, Vol.5, pp. 581-591, 1178-2013 (Electronic), 1176-9114 (Linking)

Yessine, M. A. & Leroux, J. C. (2004). Membrane-destabilizing polyanions: interaction with lipid bilayers and endosomal escape of biomacromolecules. *Adv Drug Deliv Rev*, Vol.56, No.7, (Apr 23), pp. 999-1021,

Yoo, J. W., Chambers, E. & Mitragotri, S. (2010). Factors that control the circulation time of nanoparticles in blood: challenges, solutions and future prospects. *Curr Pharm Des*, Vol.16, No.21, (Jul), pp. 2298-2307, 1873-4286 (Electronic), 1381-6128 (Linking)

Zhang, P., Rogelj, S., Nguyen, K. & Wheeler, D. (2006). Design of a highly sensitive and specific nucleotide sensor based on photon upconverting particles. *J Am Chem Soc*, Vol.128, No.38, (Sep 27), pp. 12410-12411, 0002-7863 (Print), 0002-7863 (Linking)

Zhou, T., Neubert, H., Liu, D. Y., Liu, Z. D., Ma, Y. M., Kong, X. L., Luo, W., Mark, S. & Hider, R. C. (2006). Iron binding dendrimers: a novel approach for the treatment of haemochromatosis. *J Med Chem*, Vol.49, No.14, (Jul 13), pp. 4171-4182, 0022-2623 (Print), 0022-2623 (Linking)

Zuhorn, I. S., Bakowsky, U., Polushkin, E., Visser, W. H., Stuart, M. C., Engberts, J. B. & Hoekstra, D. (2005). Nonbilayer phase of lipoplex-membrane mixture determines

endosomal escape of genetic cargo and transfection efficiency. *Mol Ther*, Vol.11, No.5, (May), pp. 801-810,

Zurn, A. D., Widmer, H. R. & Aebischer, P. (2001). Sustained delivery of GDNF: towards a treatment for Parkinson's disease. *Brain Res Brain Res Rev*, Vol.36, No.2-3, (Oct), pp. 222-229
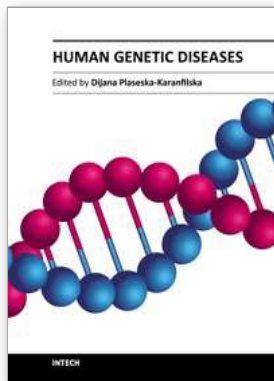
Human Genetic Diseases

Edited by Dr. Dijana Plaseska-Karanfilska

The genetics science is less than 150 years old, but its accomplishments have been astonishing. Genetics has become an indispensable component of almost all research in modern biology and medicine. Human genetic variation is associated with many, if not all, human diseases and disabilities. Nowadays, studies investigating any biological process, from the molecular level to the population level, use the â€œgenetic approachâ€ to gain understanding of that process. This book contains many diverse chapters, dealing with human genetic diseases, methods to diagnose them, novel approaches to treat them and molecular approaches and concepts to understand them. Although this book does not give a comprehensive overview of human genetic diseases, I believe that the sixteen book chapters will be a valuable resource for researchers and students in different life and medical sciences.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Janet Hsu and Silvia Muro (2011). Nanomedicine and Drug Delivery Strategies for Treatment of Genetic Diseases, Human Genetic Diseases, Dr. Dijana Plaseska-Karanfilska (Ed.), ISBN: 978-953-307-936-3, InTech, Available from: http://www.intechopen.com/books/human-genetic-diseases/nanomedicine-and-drug-delivery-strategies-for-treatment-of-genetic-diseases

INTECH
open science | open minds