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Nanoparticles as Non-Viral Gene Delivery Vectors

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Abstract

Gene therapy is being considered as a potential medical revolution. Initially, it was viewed as an approach for treating hereditary diseases, but now wide recognition of its potential role in the treatment of acquired diseases such as cancer is being envisaged. Non-viral vectors based on the use of cationic lipids or polymers appear to have promising potential, given the problems of safety encountered with viral vectors. Based on the advantages and disadvantages of existing vectors and on the hurdles encountered with these carriers, the aim of this review is to describe the "perfect vector" for systemic gene therapy against cancer. Non-viral vectors consist of a vector backbone, usually a cationic lipid or polymer able to form stable complexes with the plasmid, modified by incorporation of functional groups, and often combined with biomaterials to enhance cytocompatibility. Currently, the major drawback of gene therapy is the gene transfection rate. The two main types of vectors that are used in gene therapy are based on viral or non-viral gene delivery systems. The viral gene delivery system shows a high transfection yield but it has many disadvantages, such as oncogenic effects and immunogenicity. In the present communication, we report on a new gene delivery system based on the various nanoparticulate approaches like Cationic lipids, Cationic polymers, Gold Nanoparticles, Magnetic nanoparticles, Quantum dots, Silica nanoparticles, Fullerenes, CNTs, Supramolecular systems.

Key words: Nanoparticles, Non-viral Vectors, Gene therapy, Transfection.

INTRODUCTION

The delivery of functional genes to target cells for achieving therapeutic effect is defined as gene therapy. Gene therapy being treatment or prevention of disease by gene transfer is being considered as a potential medical revolution. However, the biological system being complex becomes an obstacle for successful gene delivery. The understanding of the molecular mechanisms involved in cancer and the development of nucleic acid delivery systems are two concepts that have led to this development. Systemic gene delivery systems are needed for therapeutic application to cells inaccessible by percutaneous injection and for multi-located tumor sites, i.e. metastases. Genes mostly nucleic acids and plasmids are delivered to supply to deficient or missing production of certain genes. The application of gene therapy in the suppression or replacement of malfunctioning genes holds promise in treatment of diseases at genetic level as well as understanding physiological roles of genes. Vectors are agents, which will protect the genetic material and transport it to the cellular or nuclear interior of the intended cell type. The non-viral gene transfer methods will enable targeting and analysis of biological effects to affected cells or tissues. The efficient ectopic expression of foreign genes is most critical for successful in-vivo manipulation experiments or therapy. Not only gene delivery but gene expression is also important for therapeutic effect. Delivery of oligonucleotides and RNA in antisense therapy aims at decreasing or inactivating target protein production causative of particular disease. Perfect gene delivery is still on the rise since many adverse effects have emerged which include cases of undesired expression of dangerous proteins or down-regulation of others leading to tumors and fatal cases.

GENE DELIVERY VECTORS:

(a) Types of gene vectors:

The important characteristics of a good vector are:

* Should replicate autonomously.
* Easy to isolate and purify.
* Easily introduced into host cells.
* Suitable marker genes to be present in the vector.
* Cells transformed with vector containing DNA insert (r-DNA) should be identifiable or selectable from

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those transformed by unaltered vector.

* A vector should contain unique target sites for as many restriction sites as possible into which DNA insert can be integrated.

When expression of DNA insert is desired, the vector should contain at least suitable control elements like promoter, operator and binding sites. Direct delivery of naked DNA proved inefficient and non-viable due to very low transfection proficiency. This is due to the biological barriers that have to be overcome by the DNA to reach its destination for expression i.e. cell nucleus. This problem has led to evolution of various gene delivery systems where the gene carrier compacts the plasmid DNA and protects it from enzymatic degradation. There are broadly two types of gene delivery vectors: viral and non-viral. Viral vectors are believed to have all tools for efficient binding to target cells, internalization and uptake into nucleus where finally DNA release occurs but now are decreasingly used owing to safety risks and hazards. So now artificial/non-viral vectors are being increasingly used due to ease of their engineering. The use of non-viral vectors allows designing of polyvalent carriers which can incorporate many functional components each with a different function. So in a way it can be said that an ideal vector should potentiate the efficiency and undermine the drawbacks of viruses.

(b) Delivery pathways and Cellular barriers:

Before initiating design of ideal non-viral delivery system, the identification of the cellular barriers to be bypassed by the vector during its journey to cell nucleus is essential.

DNA Compaction: The essential prerequisite for successful gene carrier is the ability to interact with the DNA. The half-life of naked DNA is further enhanced by compaction with vector, which protects it from plasma proteases preventing degradation. Cationic compounds can cause DNA condensation by interacting electrostatically with the phosphate groups of DNA, lipids through hydrophobic interactions. High concentrations of neutral polymers like polyethylene glycol (PEG) and polyvinylpyrollidone can collapse DNA molecules into compact particles through non-specific interactions by excluding solvent volume. [1]

Crossing the plasma membrane: After the formation of the complex, internalization has to occur in the target cell. If no specific ligand is available at the carrier surface, then there will be nonspecific in vivo release of DNA and this will be either randomly delivered to the cells by adsorption or degraded in the plasma serum. Receptor mediated pathway can be enhanced by ligand fictionalization of carrier surface. Ligands used include folic acid, transferrin and epidermal growth factor (EGF) in targeting cancer cells [2, 3, 4], RGD peptides for epithelial cells [5] and anti-CD3 ligands in targeting T-cells. [6]

Endosomal escape: After the complex crossed the cellular membrane, it is wrapped up into endosomal compartment in the cytoplasm. The endosomal compartment has to be destabilized by use of polyethyleneimines (PEI) to prevent the escape or degradation of the aggregate formed. PEI prevents lysosomal degradation and causes endosomal escape through proton sponge process. [7]

(c) *Gene therapy:*

Gene therapy is mainly classified as:

a) Germ-line gene therapy

b) Somatic cell gene therapy

a) Germ-line gene therapy:

Germ cells are modified by introduction of functional genes ordinarily integrated into their genomes. Change here is heritable i.e. passed on to later generations and now have proved to be effective theoretically in counteracting genetic disorders.

b) Somatic cell gene therapy:

*G*ene is introduced only in somatic cells especially of those tissues where gene expression is critical for health. Now this has emerged as the only feasible option leading to clinical trials for treatment of cancer and blood disorders. This is has further been divided on the basis of the end result of process into (i) Augmentation gene therapy and (ii) Targeted gene transfer.

(I) *Augmentation gene therapy* involves introduction of functional gene in addition to defective gene endogenous to the cells i.e. the modified cells contain both defective (endogenous) as well as normal (introduced) copies of the gene.

(ii) *Targeted gene transfer* involves homogenous recombination to replace endogenous gene with introduced functional gene. [8]

(d) Types of Non-Viral Vectors:

Many types of synthetic vectors have been developed in

recent times keeping in mind their aim of compacting DNA and protecting it throughout its journey upto cell nucleus.

The various approaches include:

(I) Cationic lipids
(ii) Cationic polymers
(iii) Gold nanoparticles
(iv) Magnetic nanoparticles
(v) Quantum dots
(vi) Silica nanoparticles
(vii) Fullerenes
(viii) CNTs
(ix) Supramolecular systems

(i) Cationic lipids: Cationic lipids are made up of a cationic head group attached by a linker to a lipid hydrophobic moiety. The positively charged head group is necessary for the binding of nucleic acid phosphate groups. All cationic lipids are therefore positively charged amphiphile systems. They can be classified into various subgroups according to their basic structural characteristics:

(1) Monovalent aliphatic lipids: Characterized by a single amine function in their head group, e.g. N[1-(2,3-dioleyloxy) propel]-N,N,N-trimethylammonium chloride (DOTMA), 1,2-dioleyl- 3-trimethylammonium-propane (DOTAP), N-(2-hydroxyethyl)-N,N-dimethyl 2,3-bis(tetradecycloxy-1-propanaminium bromide (DMRIE).

(2) Multivalent aliphatic lipids: Whose polar head groups contain several amine functions such as the spermine group, e.g. dioctadecylamidoglycylspermine (DOGS). Cationic lipids present three main components: a polar head group, which allows compaction of the DNA, a lipidic chain, which allows self- association through hydrophilic interaction forming micelles or liposomes; and a linker, which interconnects the two above-mentioned functional groups. Cationic lipids and DNA usually interact forming lipoplexes, which are multilayered structures where the DNA is sandwiched between the cationic lipids. [9]

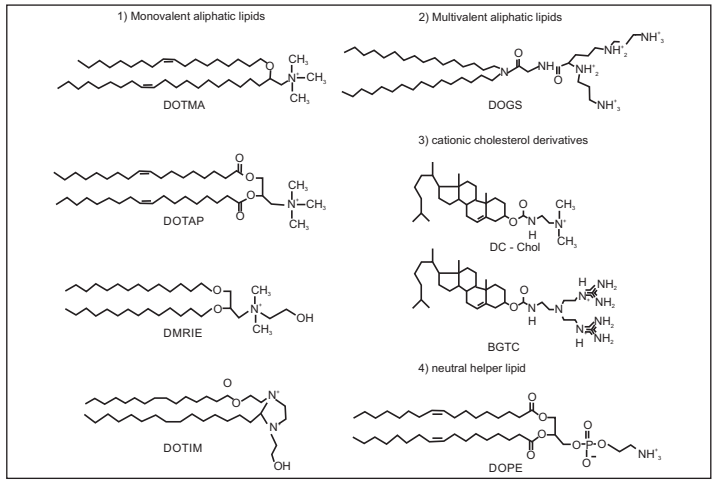

Fig. 1: Structure of current cationic lipids used in gene therapy and the helper lipid DOPE. DOTMA: N[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride, DOTAP: 1,2-dioleyl-3-trimethylamonium-propane, DMRIE: N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy-1-propananium bromide), DOTIM: 1-[2-dioleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride, DOGS: dioctadecylamidoglycylspermine, DC-Chol: [N-(N0,N0-dimethylaminoethane)-carbamoyl]cholesterol, BGTC: bis-guanidium-tren-cholesterol, DOPE: 1, 2-dioleyl-sn-glycerol-3-phosphoethanolamine

The ease of synthesis of cationic lipids and the availability of almost unlimited cationic and lipophilic groups allowed the generation of many categories based on these non-viral vectors. Kim etal described two new cationic lipids where aspartic acid or glutamic acids were used as linkers between a core of lysine (polar head) and two C14 hydrocarbon chains.[10] The lipoplexes formed by these vectors presented strong cationic surface and great stability, resulting in strong cationic surface and great stability, resulting in strong binding affinities for cell surfaces. Used in conjunction with cholesterol to aid membrane fusion, these derivatives were seen to

effectively transfecting hepatic and intra-tumoral cells both in vitro and in vivo. They can self-assemble by co-operative hydrophobic intermolecular binding at a certain concentration, forming cationic liposomes. The lipids can then bind and compact nucleic acid by electrostatic interaction between the positively charged polar heads of the lipids and the negatively charged phosphate groups of the DNA, forming cationic lipid/DNA complexes a.k.a. lipoplexes. The nucleic acid is protected from the degradation of nucleases and is able to reach the desired site of the cells. Cationic lipids especially facilitate the transfection during the early stage of the intracellular process by condensing the DNA and binding with cell membranes. Cationic lipids offer greater ease of use and efficiency with low toxicity. However, cationic lipids become cytotoxic, usually resulting in 30-40% loss in viability beyond certain charge ratio (lipid/DNA=3:1) 20. Regarding the lipoplex-mediated gene delivery, several cationic lipids were approved for clinical trials, such as DC-Chol for breast and ovarian cancer21 and DMRIE for human basal cell. Adding DNA to cationic liposomes results in either lamellar or inverted hexagonal phase structure (Fig. 2). The lamellar form is a condensed and globular structure, consisting of DNA monolayers, characterized by uniform inter-helical spacing, sandwiched between cationic lipid bilayers[11], while the inverted hexagonal phase structure consists of DNA coated with cationic lipid monolayers arranged on a two-dimensional hexagonal lattice.[12, 13] For transfection application, cationic lipids are often mixed with so-called helper lipids, like DOPE (1, 2-dioleyl-sn-glycerol-3-phosphoethanolamine) or cholesterol, both potentially promoting conversion of the lamellar lipoplex phase into a hexagonal structure, which is known to improve transfection efficiency. It is worth noting that the ratio and combination of cationic/helper lipids are important factors for transfection efficiency and toxicity.[14, 15]

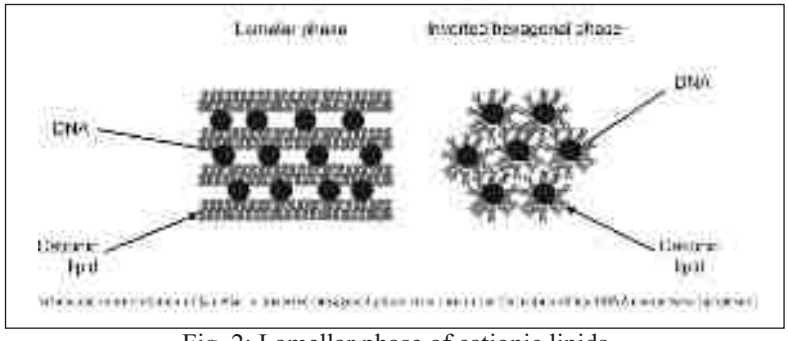

Fig. 2: Lamellar phase of cationic lipids

Taking into account the cytotoxicity at high concentrations of the cationic lipids, another approach is the employment of neutral lipids for gene delivery where Leblond etal used lipopolythioureas as non-viral vectors[16] where using thiourea lipids and a lipid containing an RGD peptide, stable complexes were formed with DNA and efficient internalization into integrin αβ expressing cells.

(ii) Cationic Polymers: Cationic polymers are usually classified in two main groups: natural polymers, such as proteins and peptides, polysaccarides, and synthetic polymers, such as polyethyleneimine (PEI), dendrimers, and polyphosphoesters. They interact with the DNA through electrostatic interaction by means of amines and/or ammonium ions. The ratio between the number of vector's amines and the number of phosphates in the pDNA is referred to as the N/P ratio, which dictates morphology and size of the complex. [17] Among natural polymers, the cationic polysaccharide chitosan has been probably one of the most widely studied non-viral vectors with numerous published trials, both in vitro and in vivo. Chitosan is nontoxic even at high concentrations and at all molecular weight ranges. However, although it shows effective nucleic acid binding and compaction, the delivery efficiency is generally low in most cell lines. Nevertheless, due to its mucoadhesive properties, chitosan/DNA polyplexes have been successfully applied to oral and nasal gene therapy. The latest strategies to improve its transfection ability comprise the synthesis of novel chitosan derivatives, such as

aminoethyl- chitin (ABC) [18], thiolated- chitosan [19], chitosan methoxy poly- ethylene glycol- cholesterol (LCP- Ch) [20] and low molecular weight alkylated chitosans [21]. To overcome intracellular barriers, such as crossing the cell membrane, recently chitosan was conjugated to folic acid (FA) for targeting tumor cells allowing folate- receptor mediated endocytosis. [22,23] Liu et al. reported use of RNA (siRNA) to silence unwanted genes and so contribute to defense from viral infections. [24] Among the polycations used for gene delivery, PEI is also a very interesting candidate and another of the most extensively investigated carriers, due to its compacting skills and its buffering ability, which allow having in some cases transfection efficiencies comparable to those of viral vectors. The physicochemical properties of PEI-based polyplexes and structure- function relationships were recently reviewed by Kissel. [25] The strategy of "PEGylation" (formation of PEG-PEI conjugates in order to improve biocompatibility) has been widely used in gene delivery; however, other substitutes to PEG linkages may lead to new alternatives. Low cytotoxicity and transfection were observed at pH 7.4 by Sethuraman et al. [26] when pre-condensed PEI/DNA complexes were coated with PSD. However, extra cellular matrix of tumors have a lower pH and this system at pH 6.6 presented high cytotoxicity and transfection, probably caused by PSD-b-PEG detachment and increased interaction between the PEI and the cells. Biodegradable polymers based on poly (aminoesters) are another promising class of vectors because of their low cytotoxicity due to cleavage of the ester bonds by the plasma enzymes. Diverse poly (B-aminoester) polymers have been synthesized and tested for gene therapy. [27, 28] Zugates et al. [29] bound an RGDC peptide to the polymer for targeting endothelial cells. Reaching transfection levels comparable to those of PEI in human hepatocellular carcinoma cells. The same authors previously described the synthesis of a natural polymer (B-aminoester)s and tested these compounds as vectors for cardiovascular diseases and cancer. About four decades ago, it was discovered that coating DNA with Diethylaminoethyl-dextran (DEAE-dextran) would allow DNA to transit across the cell membranes. Currently, commonly used cationic polymers include poly (L-lysine) (PLL), polyethylenimines (PEI), polyamidoamine dendrimers (PAMAM), and chitosan. Among them, PEI and PAMAM are the most frequently used because of the excellent transfection efficiency. It has been shown that (PAMAM) (< 5 nm) can selectively target tumor cells when loaded with an anticancer drug. The main disadvantage of these polymers is their pronounced toxicity. Cell culture experiments demonstrated that poly (glucaramidoamine)s retain low toxicity profiles. The polymers D4, G4 and m4 presented the highest delivery efficiency, especially G4. This is probable due to the fact that G4 presents the highest DNA binding affinity, as indicated by competition assays with heparin, which mimic the interactions between the polyplexes with glycosaminoglycans on the cell surface.

(iii) Gold nanoparticles: Gold is a reasonably inert metal, it does not oxidize at temperatures below its melting point; it does not react with atmospheric O_2; it does not react with most chemicals. These properties make it possible to handle and manipulate samples under atmospheric conditions. On the other hand, gold nanoparticles can be easily synthesized by citrate reduction in an aqueous solution or from an organic solution as discussed in the previous sections. Sizes of the nanoparticles can be tuned reliably and routinely from 1 nm to 200 nm with <10 % size dispersity. They are also exceptionally easy to functionalize. Gold nanoparticles attracted much attention in the last decade due to their ease of preparation and to the possibility of almost unlimited surface fuctionalisation. The group of Rotello dedicated much effort to studying the interaction between cationic gold NPs and DNA. They started studying the inhibition capability of tetraalkylammonium legends of gold NPs showing that these clusters were able to completely inhibit transcription by T7 RNA polymerase in-vitro.[30] They later demonstrated that DNA binding due to these nanoparticles could be reversed by complex contact with glutathione at intracellular concentration, thus creating an efficient system for a controlled release of DNA.[31] The most efficient NPs studied were eightfold more effective than 60 kDa PEI in transfecting 293T cells in the presence of 10% serum and 100 μM chloroquine. Recently, they built positively charged gold NPs bearing a photoactive o-nitrobenzyl ester linkage, which allows a controlled release of the DNA upon UV light irradiation.[32] The group of Nikome functionalized gold NPs with 2-

aminoethanetriol molecules obtaining a very simple gene delivery vector.[33] Addition of the amphiphilic N-dodecy1-PEI during complex formation yielded a ternary system which was able to transfect one order of magnitude more efficiently than commercial 25 kDa PEI. Complexes with a diameter of a few hundred nanometers and a charge ratio of approximately 8 performed best in the transfection experiments, achieving efficiencies comparable to those of commercial gene delivery agents such as calcium phosphate and lipofectamine, although with lower cytotoxicities.[34] In-vitro transfection experiments performed by Salem etal on human embryonic kidney mammalian cell limes (HEK293) yielded a fourfold increase compared to naked DNA, and a further twofold increase when the nanorods were conjugated to transferin. Preliminary in vivo experiments in mice also showed a noteworthy increase in luciferase expression either by particle bombardment (830 times higher than background) although with different transient expression depending on the method used. [35]

Polymerase chain reaction was used to amplify pEGFP-N1 DNA which was then covalently attached to the gold nano-rods by means of a thiol linkage present on the plasmid. Upon femtosecond near infrared (NIR) irradiation the conjugates released the DNA and GFP expression was observed in HeLa cells in the area locally exposed to the laser. The system showed lower susceptibility to degradation by nucleases compared to the corresponding free ODN, high cellular uptake, and delivered the genes more efficiently compared to other commercial reagents.

Gold nanoshells are a new class of nanoparticles with highly tunable properties. They are concentric sphere nanoparticles consisting of a dielectric (typically gold sulfide or silica) core and a metal (gold) shell. Potential biomedical applications of the nanoshells include immunoassays, modulated drug delivery, photothermal cancer therapy and imaging contrast agents. For example, composites of thermally sensitive hydrogels and optically active nanoparticles have been developed for drug delivery. In this method, metal nanoparticles release their drugs that are held in the hydrogel matrix such as methylene blue and proteins of varying molecular weight, into the targeted location using an external exciting source like an infrared light or a magnetic field. A possible problem is that the excretion of inorganic nanoparticles and their accumulation in the cell may harm the cell growth. Due to the high chemical stability, inorganic nanoparticles cannot be dissolved easily in the cell; and excretion is difficult due to their large size.[8]

(iv) Magnetic Nanoparticles: Much attention has been devoted to the development of magnetic NPs due to the fact that these metallic clusters possess great potential in drug delivery and gene therapy. One of the primary factors affecting efficiency in gene delivery is the low amount of DNA that manages to reach the target cells. To this regard, the development of non-viral vectors which can be directed selectively toward specific cells could reduce noteworthy the amount of plasmid which do not reach the desired site, improving significantly the chance of gene expression. Magnetic NPs can be guided through the use of an applied magnetic field to specific tissues, organs or possibly even into cells, which is the basic principle of magnetofection, allowing to reduce considerable the dose of DNA to inject and the time necessary to reach the desired target cells, enhancing significantly gene expression efficiency. [36, 37, 38] The most used magnetic clusters are superparamagnetic iron oxide NPs (IONPs), mostly magnetite (Fe_3O_4), although many other metals, such as Co (II), Mn (II), Cu (II), Ni (II), Cr (III) and Gd (III), could be suitable for such purpose. However, for in vivo experiments iron oxides are the most appropriate magnetic particles since they have already been extensively used in magnetic resonance imaging and their pharmacokinetics and toxicity studied.[39] These NPs can be easily synthesized and coated with a layer of biocompatible polymer which can be further functionalized covalently or noncovalently with biomolecules to confer to the metallic cluster the desired additional properties. No significant differences were observed in transfection efficiency between polycationinc or polyanionic coatings, nor when different types of polycation (PEI of various molecular weights or DEAE dextran) or polyanion (poly(aspartic acid), poly (Maleic acid), poly(acrylic acid), or phosphate- functionalized starch) were compared.[36] The salt- induced aggregation method was used to form the complexes with quantitative association. By means of electron microscopy and transfection experiments in the presence of various inhibitors that operate at different

steps of endocytosis, it was demonstrated that the mechanism of cellular uptake of these systems is analogous to that of PEI polyplexes. Although mainly unspecific endocytosis was observed depending on the cell line. However, the main role of the vector- DNA complex nears the cell surface, thus facilitating the chance to be internalized, without interfering in the uptake process.[40] Human umbilical vein endothelial cells (HUVEC), which are well known to be resistant to transfection of pDNA, have been also transfected by this method enhancing expression of luciferase gene by a factor of 360 compared to various conventional transfection systems. Contransfecting with PEI, even confluent HUVEC cells could be efficiently transfected. In another study magnetofection was used to deliver p22phox- specific antisense oligodesoxynucleotide (ODN) to endothelial cells allowing investigating the role of this NAD (P) H-oxidase subunit in Oxygen production. [41] Several standard transfecting agents were used in conjunction with IONPs, the most effective being Effectene, which was able to transfect up to 90% of cells. Application of a magnetic field did not augment further the in vivo outcome, probably due to poor technical power/control of the magnetic field. Compared to the free vector, in vitro experiments showed that just 1% of magnetic vector was necessary to obtain similar transduction levels, while higher levels were reached in *in- vivo* experiments. Application of a magnetic field didn't increase the *in-vivo* outcome when haemaglutinating virus of Japanese type (HVJ-E) was used to aid gene transfer to magnetic NPs by cell fusion. This was thought to occur due to poor technical control over the magnetic field. [42] Commercially available streptavidin-coated magnetic IONPs were bound to biotin- labeled TAC and easily purified using a magnetic column. Addition of an outer lipidic shell slightly improved gene expression, although lipid-free NPs were able to successfully transfect cells in vitro without visible toxicity.[43] Finally, it can be seen that the main advantages of magnetically enhanced nucleic acid delivery-magnetofection –are the rapidity of the delivery, just a few minutes compared to other common transfection methods, and the site –specific vector targeting in vivo, which allows saving considerable amounts of material.[36] On the other hand, exploiting the full potential of the technique requires set up of specific procedures. Nevertheless, this relatively new system holds great potentiality and, although just in its infancy, it already showed how it can enhance gene transfer and expression.

(v) Quantum Dots: Quantum dots (QDs) are semiconduction nanomaterials that, due to their physical size and composition, present bright fluorescence, narrow emission, broad UV excitation, and high photostability with numerous advantages over traditional organic dyes.[44] This characteristic, additionally with the possibility to bio- functionalize them, offers great potential for biological and medical application, mostly for imaging and sensoring. Only 15% of the nanocrystals were found inside the nucleus within 24 hrs, while 85% was located in the perinuclear region, probably because of the overall size of the NPs, often too big to pass through the nuclear pores. Furthermore, the system showed to have low cytotoxicity and, thanks to the QDs fluorescence, the researchers were able to visualize the movement of the NPs inside the cytoplasm toward the nucleus.[45] Cheng et al. prepared fluorescent silica nanotubes (fNTs), by incorporating CdSe/ZnS core-shell quantum dots, functionalized with 3-(aminopopy1) trimethoxysilane (APTMS), to generate a polycationic surface.[46] The (fNTs), with average outer diameters of about 200 nm and 2Um long, were incubated with the pDNA and in vitro experiments with COS-7 cells showed that approximately 10%-20% of the cells expressed GFP.

(vi) Silica Nanoparticles: Silica is a major component of many natural materials, from sand to glass, and it has been used extensively for a long time. More recently, silica has been used in biomedicine due to its relative ease of functionalization. The most commonly used method to exploit silica for gene delivery is by functionalizing the surface of the NPs with amino-silicanes. The group of Lehr showed that commercially available silica NPs functionalized with N-(6-aminohexy1)-3-aminopropyltrimethoxysilane are able to transfect efficiently Cos-1 cells with very low toxicity.[47] More recently, the same group used amino-hexy1-aminopropy trimethoxysilane (AHAPS) functionalized silica NPs to transfer pEGFP gene in vivo in the mouse lung. [48]

He et al. used a similar approach but they directly synthesized positively charged amino-modified silica NPs.[49] They showed an efficient protection method which

could have many potential applications in biotechnology. These NPs were able to down-regulate significantly c-myc mRNA by delivering antisense ODN in HNEL and HeLa cells in serum-free medium, although its delivery efficacy decreased in the presence of serum-containing medium due to interaction between the NPs and serum proteins. The organically modified silica (ORMOSIL) NPs encapsulating fluorescent dyes and surface-functionalized by cationic amino groups were able to protect the pDNA from enzymatic degradation and to successfully transfect COS-1 cells.

(vii) Fullerenes: Fullerenes are water-insoluble carbon molecules. The first example of a functionalized fullerene as a gene delivery vector is from Nakamura et al. who functionalized a fullerene with two diamine side chains.[50] The fullerene showed transfection efficiency comparable to that of commercial reagents and the morphology of the aggregates indicated that the complex is taken up by cells through phagocytosis. However, the poor solubility of the fullerene derivative in the cell medium required addition of dimethylformamide (DMF), thus raising big toxicity problems and consequent very low cell viability. Very recently, the same group reported the synthesis and gene delivery ability of a library of aminofullerenes and studied key elements which influence the fullerene-mediated transfection efficiency. [51] Among these, the cationic charge of the fullerene derivative and the medium for the preparation of the fullerene/DNA complexes, which in turn control the size of the complexes.

(viii) CNTs: Carbon nanotubes, which are basically cylindrical fullerenes, also showed to have great potential for biological applications.[52] Pantarotto etal. functionalized CNTs with an amino-terminal oligoethylene glycol chain. These functionalized CNTs (f-CNTs) present low cytotoxicity and are able to associate with DNA and efficiently deliver it to CHO cells with gene expression levels up to ten times higher than those achieved with DNA alone. In another report by the same group, single-walled and multiwalled CNTs were functionalized with ammonium groups and lysine.[53] The study showed that, as expected, increasing charge ratios lead to greater amounts of DNA condensed; however, gene delivery experiments showed that all three systems were able to transfect human lung carcinoma cell line (A549) demonstrating that very efficient DNA condensation might not be necessary for effective gene transfer. Lu etal. reported the translocation of RNA polymer poly (rU) into breast cancer cells (MCF7).[54] By using radioisotope labeling assay and confocal microscopy, they were able to confirm the successful gene delivery and the RNA was found inside the cellular and nuclear membranes. Furthermore, the CNTs showed to be highly biocompatible with negligible cytotoxicity. Cai etal. used nickel-embedded magnetic CNTs which align to the magnetic flux when exposed to a magnetic field, allowing hitting perpendicularly the cell membrane.[55] This method, called "nanotube spearing", allowed reaching successful gene delivery into nondividing cells, such as primary B cells and neurons, which are notoriously hard to transfect, reaching levels of transfection equivalent to those of viral vectors. Gao etal. also used amino-functionalized multiwalled CNTs to deliver pEGFP-N1 pDNA to HUVEC and A375 cell cultures.[56] The plasmid was successfully delivered although efficiency was lower compared to Lipofectamine 2000, although the cytotoxicity of the amino-CNTs was much lower compared to that of the commercial vector.

(ix) Supramolecular systems: The group of Aoyama demonstrated that highly saccharide-functionalized porphyrin and calix[4] resorcarene derivatives exhibit a remarkable saccharide specificity toward hepatic cells. [57] In a later study, they also showed that these type of masked hydrophobic molecules assemble with the plasmid DNA to form glycoviruses which are able to efficiently transfect HeLa cells.[58] Ungaro etal. also applied a similar approach by functionalizing a series of calix[n]arenes at the upper rim with guanidinium groups and alkyl chains at the lower rim.[59, 60] These compounds efficiently condensed the DNA and, in some cases, were able to promote cell transfection depending on their structure and lipophilicity. However, the most important finding of the study was the better understanding of the factors affecting DNA condensation and cell transfection depending on the structure and the conformation of the nonviral vector. Guanidinium moieties were also used by Fernandez- Carneado etal. who showed that tetraguanidinium oligomers are able to translocate through HeLa membranes even more efficiently than Tat

or Antp peptides, finally accumulating in mitochondria.[61] A similar approach has also been exploited by Li etal.[62] They synthesized a class of nonviral vectors based on cationic polyrotaxanes formed by multiple oligoethylenimine-grafted-cyclodextrin (CD) rings blocked on a polymer chain. These cationic supramolecular vectors could efficiently condense DNA into small nanoparticles giving low cytotoxicity and good gene transfection efficiency.

CONCLUSION:

As described in this review, several non-viral vectors have been developed for DNA delivery. The transfection efficiency may depend on several factors such as chemical structure of polycations, size and composition of complexes, interaction between cells and complexes and the cell type. One such cationic polymer, namely chitosan, is considered to be one of the best candidates for DNA delivery. A number of in vitro and in vivo studies showed that chitosan is a suitable material for efficient non-viral gene therapy. Nanoparticle-mediated gene delivery will permit the combination of gene therapy with traditional approaches like chemotherapy. Non-viral vectors are now being researched upon due to the limitations and several disadvantages seen in viral vectors. Nanotechnology has shown that it can enable bridging up the post human future with the advent of nanorobotics. Calls for tighter regulation of nanotechnology have occurred alongside a growing debate related to the human health and safety risks associated with nanotechnology. Furthermore, there is significant debate about who is responsible for the regulation of nanotechnology but it can be said that nanoparticles have opened up the new frontiers of innovative inventions. It is thus necessary to carry on tremendous biocompatibility studies for each new system in each application thus researched upon.

Table No. 1: Comparison of viral vectors, non-viral vectors and nanoparticle vectors

Features	Viral vectors	Non-viral vectors	Nanoparticle vectors
Efficiency	High	Low	Can be high
Synthesis and Modification	Function determined by viral structures and not easily modified	Hard to incorporate multiple functions	Easy to incorporate different functions on a single particle
Immunogenicity	Elicit strong immune response	Can be controlled	Can be controlled
Size	Size restricted (30-100mm)	Individual dendrimers (<2 nm) Polymers (> 50 nm) Liposome (> 20 nm)	Size tunable from 1 nm to 200 nm

Table No.1 highlights comparative study of the features of nanoparticle vectors with regard to conventional viral and non-viral vectors.

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