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Case Report

Nanomaterials as transmitters of non-viral gene vectors: A review

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ABSTRACT

With the rapid development of nanotechnology in the recent decade, novel DNA and RNA delivery systems for gene therapy have become available that can be used instead of viral vectors. These non-viral vectors can be made of a variety of materials, including inorganic nanoparticles, carbon nanotubes, liposomes, protein and peptide-based nanoparticles, as well as nanoscale polymeric materials. They have as advantages over viral vectors a decreased immune response, and additionally offer flexibility in design, allowing them to be functionalized and targeted to specific sites in a biological system with low cytotoxicity.gene therapy keeps hopes a life for the treatment of a wide range of diseases such as cancer, nano particles are now known as promising carriers for the effective and safe vectors of genes to specific cells or tissues. This could provide alternative therapies for conventional approaches that use viruses as gene carriers. The expression of genetic material such as DNA, RNA into cells and tissues has raised considerable hopes for therapeutic and diagnostic purposes. But getting nucleic acids into the cell also faces challenges. These challenges are less for non-virus carriers as a gene and drug vectors method than for viral or free vectors and are therefore considered less risky and more appropriate. of expanding nonverbal nano carriers, we will look at a few of these nano carriers, penicillin, PEI, PLGA, silica, block copolymer, Quantum dot, gold nano particles, and common carbon nano tubes. Problems include the use of nano particles such as polymer nano particles, liposomes, solid lipid particles, in targeted gene vectors will be investigated. Gene-based therapy is the intentional modulation of gene expression in specific cells to treat pathological conditions. This modulation is accomplished by introducing exogenous nucleic acids such as DNA, mRNA, small interfering RNA (siRNA), microRNA (miRNA) or antisense oligonucleotides. Given the large size and the negative charge of these macromolecules, their delivery is typically mediated by carriers or vectors. In this Review, we introduce the biological barriers to gene delivery in vivo and discuss recent advances in

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material sciences, nanotechnology and nucleic acid chemistry that have yielded promising non-viral delivery systems, some of which are currently undergoing testing in clinical trials. The diversity of these systems highlights the recent progress of gene-based therapy using non-viral approaches.

1. Introduction

Gene therapy has the potential to cure human diseases caused using defective genes. Some of these diseases include sac fibrosis, macular degeneration, Parkinson's disease, and virus cancers [1–5]. Gene therapy has also been used to express different genes or to turn them off in animal tissues such as the central nervous system, lungs, liver, etc [6–10]. The effective development of gene therapy involves the efficient vectors of therapeutic genes to the cell to displace or silence defective disease-causing organisms in humans [11–16]. Virus vectors similar adenovirus and retrovirus are commonly occupied in gene therapy due in their high efficiency in gene delivery [17–22]. However, some problems have led to a review of the use of virus vectors in human clinical trials. Recently, non-virus particles have received widespread attention in gene therapy [23–27]. This is because they can overcome a variety of toxicities caused by virus delivery [28–32]. Some common non-virus vectors that allow genetic material to pass-throng cellular barriers are discussed in this article. Gene therapy as A potential method for the treatment of genetic disorders and others malignancies has attracted very regard in recent years [33–38]. In DNA plasmid gene therapy (plasmids are usually in the form a circular double-stranded DNA molecule (although there are linear types of plasmids that are replicated independently of the nucleus) to express specific therapeutic Proteins or oligonucleotides to silence the gene that causes disease (antisense therapy) are used [39–43]. The main importance of pure DNA plasmid is for the development and production of vaccines that a small amount of Protein can lead to the desired immune response [44–48]. Because of the degradation of DNA by serum nucleases, Pure DNA is rarely suitable for the vectors of genetic material in virus. Therefore, a carrier system is usually recommended That is biocompatible and immunogenicity [49–53]. (non-immunogenic), protects DNA against reticuloendothelial system (RES) agents and is specific to the target cell or tissue [54–57]. The nano particle process is expected to enter the endocytosis cell, so the carrier system must be able to escape the endosome and deliver DNA to the nucleus [58–62]. As shown in Fig. 1, a fine machine should include a difference of building blocks for definite behaviors. Clinical applications of genotypes haven't grown significantly because of the insecurity within body liquids their down tissue absorption due unto their above relative molecular mass, macromolecule pregnancy [63–67]. Therefore, it is need to style an appropriate delivery machine which will deliver DNA or oligonucleotide strand to the target. Two general approaches to accomplish this goal [68–73]: 1. Use of virus vectors, 2. Non-virus vectors. Despite the high efficiency of virus vectors for transmitting DNA to a wide variety of cells, there are major problems, Including the danger of safety answer to virus vectors, as well as the Possibility of accidental mixing of the virus vectors with the wild-type virus [74–80]. So, thither is a necessity for a more reliable carrier to deliver DNA [48–50]. This carrier must have the surface charge density and hydrophobicity required to Interact with the lipid components of the cell as well as the DNA [81–85]. Due to the negative Intrinsic charge of DNA (due to The Presence of phosphate structural groups), Positively charged materials are usually used as carriers to deliver DNA so that the carrier can be attached to DNA using electrostatic interaction (Fig. 2). Cationic Polymers, Cationic Phospholipids Are the two main types of non-virus gene delivery carriers currently under Investigation [86–91]. Due to the positive surface load, both kinds react electrostatic via the negative DNA, combine [92–94]. Despite the easy construction of lipid compounds, Low gene vectors and toxicity of most of them Limit their use [95–98]. Cationic Polymeric Compounds are often more stable than cationic Lipid compounds, but generally have lower gene vectors than virus carriers [99–102]. These carriers make the Polyelectrolyte with the combined DNA and Protect it from nuclease degradation [103–106]. These Polymeric vectors have good adaptability and structural variability that allow the attachment of specific components to target the vectors in gene expression Through specific receptors [107–110]. Studies have been Performed on other nanostructures for DNA vectors, A: targeting the delivery system for specific cells, B: vectors from cell membrane, C: cell absorption, setback in endosomes, D:

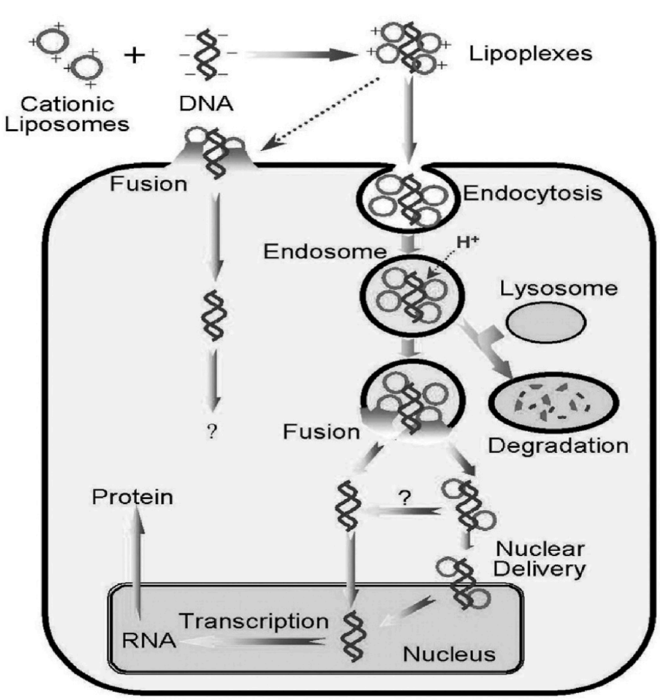


Fig. 1. The imager of the biological barriers than the gene vectors carrier must overcome. Figures reproduced with permission from Ref.[117].

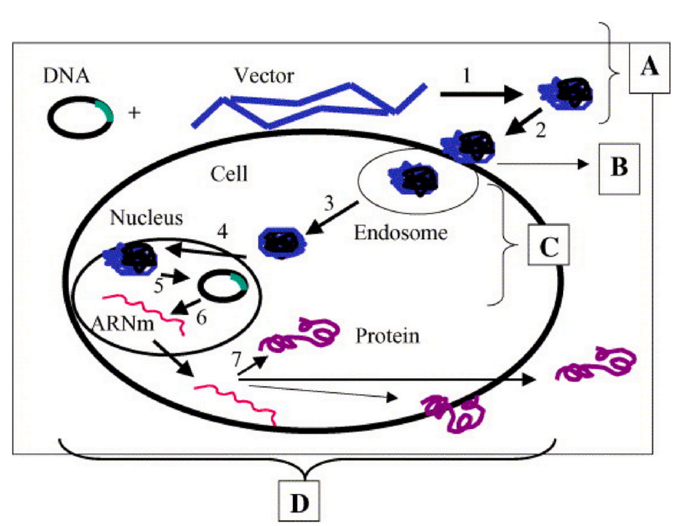


Fig. 2. Schematic of the process of interaction of a positively charged polymer carrier with a negative DNA. Figures reproduced with permission from Ref. [118].

Intracellular vectors of Plasma DNA to nucleus [111–115,116].

2. Nanoparticles for gene and drug vectors

Speiser et al. [116,119] for the first time created colloidal particles less than 1 μm in diameter that contained macromolecular compounds. Since then, much work has been done in using nano Particles the arrival of medicine and gene delivery. Initially, most nano Particles were designed to carry vaccines and anti-cancer drugs [120–124]. However, the use of these carriers for the Possibility of using drugs and vaccines orally and jocularly is currently under investigation [125–129]. Drugs or biologically active molecules are trapped, encapsulated in NPTS, chemically attached to NPTS or absorbed to the Particle surface [130–134]. The choice of the appropriate method for The Preparation of the drug carried by the nano Particle depends on the Properties of the drug and the nano particle [135–138]. Two different kinds of systems with different Internal structures are Possible [139–144].

1. Matrix-type systems consisting of the entanglement of oligomers, Polymer units known as nano spheres or nano particles [145–148].
2. Storage systems that contain a core coated with a Polymer wall and are defined as nano capsules [149–154,155].

3. Non-virus nanoparticles being studied and expanded

3.1. Chitosan

Chitosan's are the very natural amino Polysaccharide found in the cell wall of fungi, the hard skeletons of crustaceans such as crabs, shrimp, as well as in Insects [156–160]. It is estimated that its annual Production is as much as cellulose. Chitin is of great Interest, not because it is an available source, but because of Its very high potential in virus applications [161–165]. Chitosan is an amino Polysaccharide obtained from the distillation of chitin under alkaline conditions and is very similar to cellulose (Fig. 3). Chitosan is a non-toxic biocompatible Polymer that has many applications in drug delivery, more recently, In gene delivery [116,119].

Used chitosan and chitosan depolymerized oligomers to deliver the Plasmid in the virus. The resulting solution is stirred vigorously for 180–300 seconds, then left at room temperature for 1800 seconds to Combine the Particle with the Plasmid well [167–170]. Gene Vectors was Performed in virus with Cos-1 Cells and in, vivo studies were Performed on intestinal tissue [171–173]. High levels of gene expression were observed in the chitosan-Plasmid Formulation relative to Pure DNA in the upper small Intestine. In this Study, the Parameters affecting the Particle size and stability of the carrier System, chitosan molecular weight, Plasmid Concentration and Charge Ratio were determined

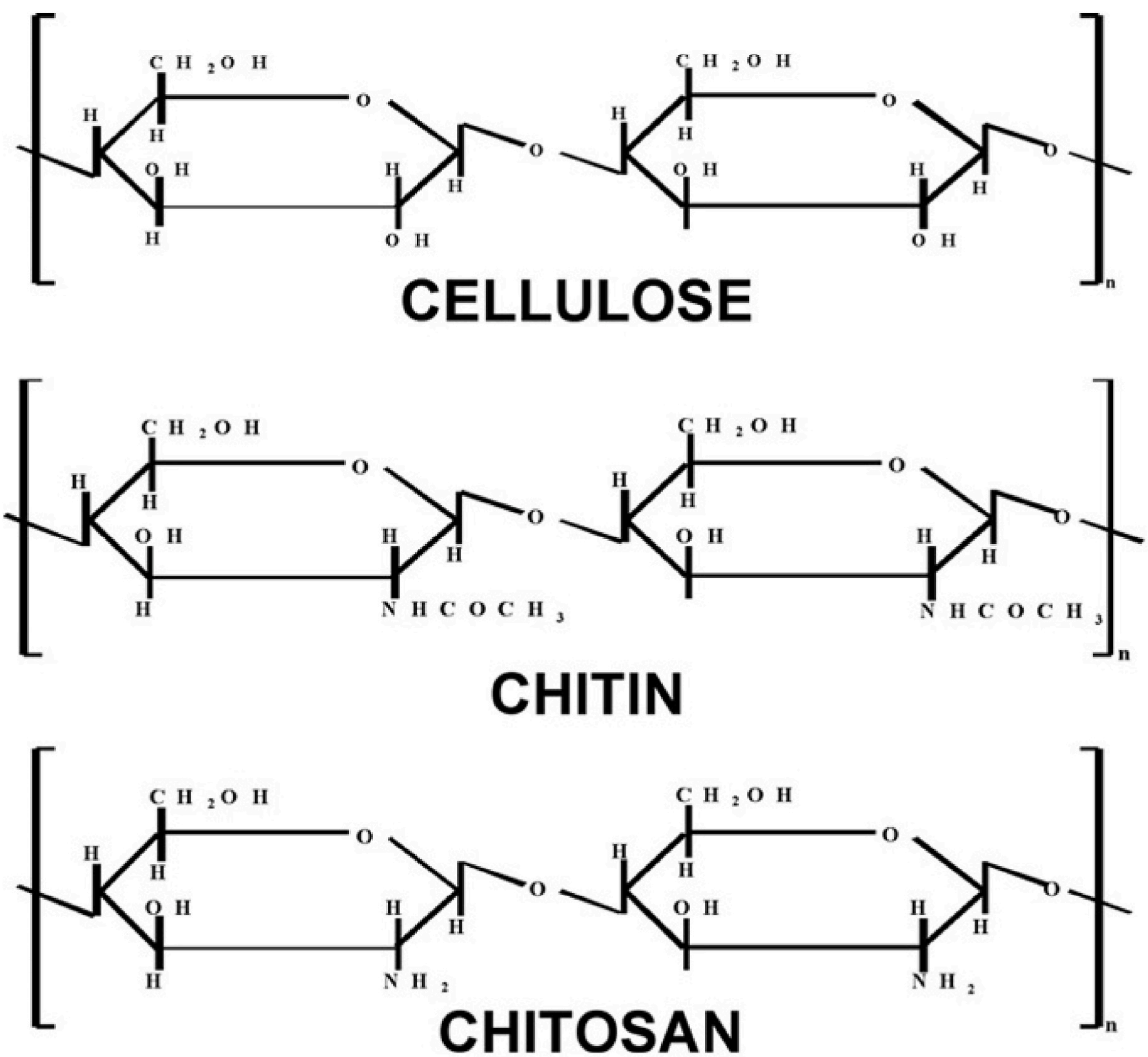


Fig. 3. Structural Similarities Between Cellulose, Chitin, and Chitosan. Figures reproduced with permission from Ref. [166].

[113–115,120,121,174–178]. The Combination of High molecular weight Plasmids and Chitosan Was shown to be more Stable against Salt and the challenges Posed by Interaction with Serum [179–182].

They examined Parameters affecting Particle Size and the Physicochemical Properties of nano Particles and demonstrated DNA Protection by chitosan [183–187]. They also Investigated PEGylating Chitosan nano Particles (nano Particles Coated with Polyethylene Glycol Polymer) (Fig. 4) to Improve storage stability as well as Formulation efficiency [116,119,188–191]. The tissue distribution of chitosan nano Particles containing DNA and chitosan nano Particles containing Pelleted DNA was Investigated and it was observed That the Clearance of Pelleted nano Particles was much slower in the First 15 minutes. It should be noted that the efficiency of gene vectors Also Depends on the Type of Cell [192–196]. Sato and his Team Found that the Gene Vectors efficiency of Chitosan depends on the molecular weight, pH of the culture medium, Serum, and Stoichiometry of the Designed System [117, 155,197–200]. Since Then, various types of lipid compounds have been used as carriers of gene vectors. Due to Their amphiphilic nature, these lipids easily Form micelles in water and Interact with DNA rapidly and efficiently due to Their Positive charge [201–205]. Cationic liposomes are Prepared by evaporating an organic solvent from a mixture of Cationic lipids and then coating the resulting lipid film in aqueous medium under high stirring Conditions, resulting in the formation of multi-layer vesicles (MLVs) [206–212]. Single-wall Vesicles are also obtained by sonication or extraction of multi-wall vesicles. Adding DNA to Cationic Liposomes Causes Dramatic Changes in liposomes and DNA [213–217]. This changes the Initial Structure of the Liposome and creates an adapted structure. The reported results show that changing the surface Characteristics of liposomes Improves the biological distribution as well as the targeted delivery of DNA to liposomes [218–222]. The structure of Some of the cationic lipids used in DNA delivery is shown in Fig. 5. They made PCL by adding Polyethylene imine Polycation (Polycation liposome system (PCL)) To the liposome [223–228]. The resulting PCL Does not require Phosphatidylethanolamine or cholesterol as components of a normal liposome. The researchers also Investigated by the effect of Polyethylene mine (PEI) %wt. on gene delivery in This method. Their results showed that Low molecular weight Polyethylene mine (PEI) was more effective than extra molecular weight Polyethylene mine (PEI) in virus gene delivery. other researchers have shown that the method is effective in vivo gene delivery [229–234].

3.2. Solid Lipid Nanoparticles (SLN)

They Found that Solid Lipid Nanoparticles (SLN), in addition to Drug Delivery, could also be a good Carrier for plasmid DNA Vectors [237–244]. This group Produced highly Cationic Solid fat nano Particles with a zeta Potential of more than +40 and a Size of 100 nm Through the hot homogenization Technique [245–249,250–254]. They Found that solid fat nano Particles fused together after binding to Plasmid DNA, producing larger Particles measuring between 300 and 800 nm (Fig. 5). The gene vectors efficiency of this Carrier has been demonstrated in virus in the Cos-1 cell line [249,255–262].

3.3. Polyethylene mine and penicillin

Structure Penicillin (Fig. 6) shows. It has high biodegradability and, of course, high toxicity, which limits its in-body use. “Preliminary studies show that if the right molecular weight” “Polyethylene mine (PEI) (Fig. 6) has been the first carrier " “PLA and PLGA particle synthesis methods are considered and, in this method,”, 100 nm can be made that can be removed by the cell in small quantities [160–162, 201-2254-260]. The reason for this is the lack of amine groups at the particle level (low positive charge). However, by using cellular targeting groups as well as endosomal lysis agents such as Chloroquine, this defect of low gene Vectors perception can be eliminated. In an effort to improve gene transfer efficiency and reduce toxicity [263–267]. In this method, PEG first binds to Penicillin and then interacts with DNA, and then binds to the Phosgene Peptide [118,268–272]. The resulting Particle has a positive charge and as a result the resulting particles have good stability and of course the use of PEG and Peptide reduces toxicity and Increases

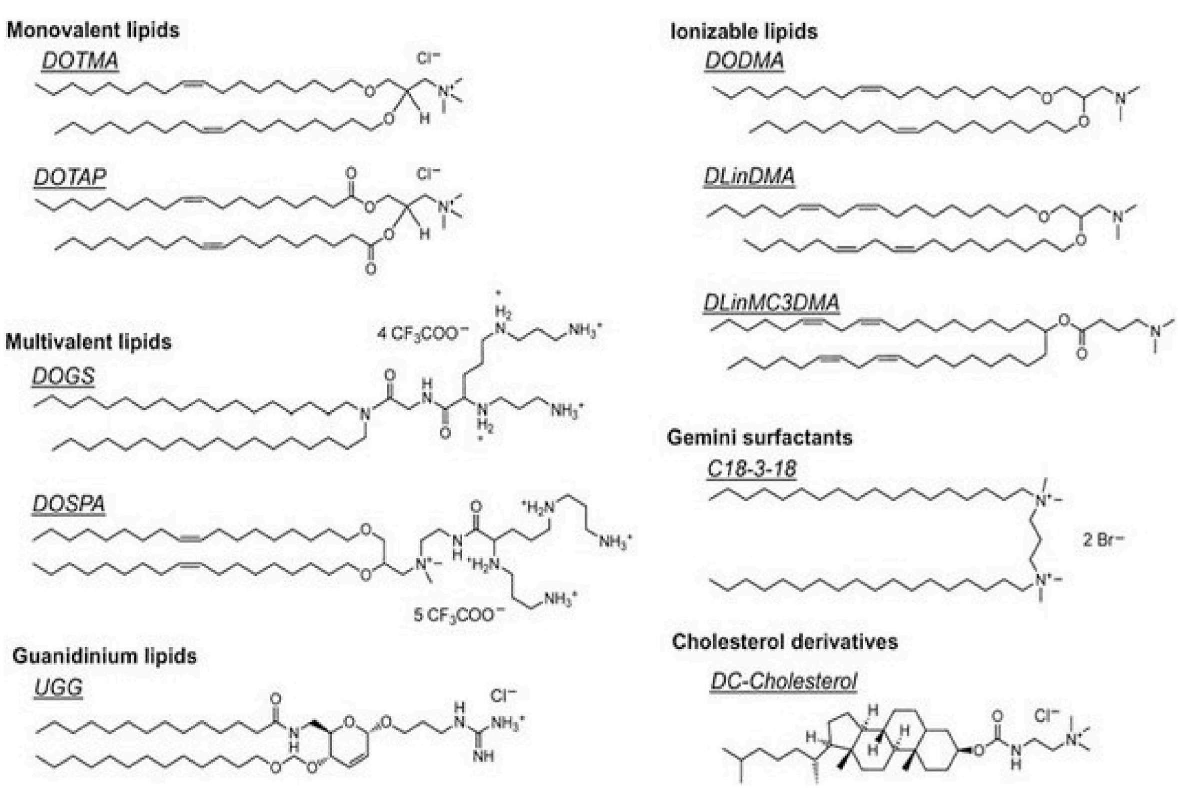


Fig. 4. Some Cationic Lipids Used in gene delivery. Figures reproduced with permission from Ref. [235].

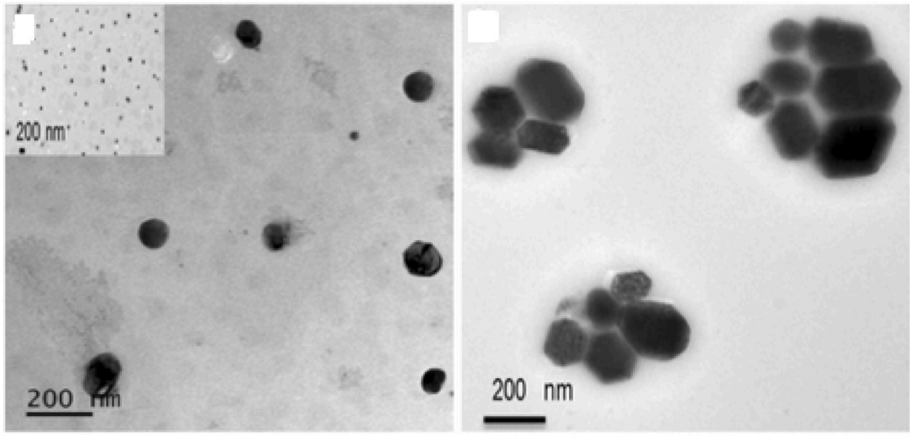


Fig. 5. Solid Lipid nanoparticles (SLN)with plasmid DNA (upper half) made from a combination of several smaller particles. A small particle can be seen at the bottom of the image. Figures reproduced with permission from Ref. [236].

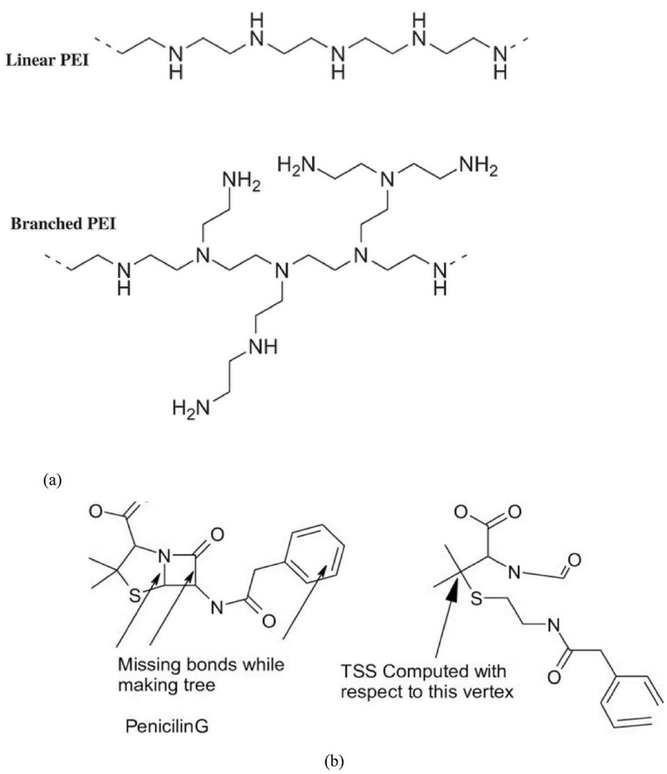


Fig. 6. Structure of (a) PEI [274] (b) Penicillin. Figures reproduced with permission from Ref. [273].

the Improvement of gene Vectors [163–165,166,235,236,273].

Polyethylene mine (PEI) (Fig. 6) has been the first carrier of gene Vectors and has been the subject of many studies. of course, the toxicity of Polyethyleneimine is the biggest drawback of this carrier [167,168, 223–225,274–278].

Sagara and Kim [116] reported the use of a Combination of Galactose, Polyethylene Glycol, and polyethylene mine (Glc-PEG-PEI) for Gene Delivery to Hepatocyte cells, and showed better Transmission of this system than similar PEI carriers [116,119]. They introduce this system as a suitable system for liver gene delivery.

Rudolph et al. [169] report the use of PEG-coated PEI to Deliver DNA to the lungs through the use of nebulizers or intratracheal injections. The author reports that the use of high molecular weight PEG reduces the efficiency of gene vectors. This may be due to the PEG Space barrier to the carrier interacting with the particle [169–171,226–229].

3.4. Poly (lactide-Co-glycolide)

Various polymers Have Been Used for Drug Delivery and gene Delivery applications, but PLA = poly (lactic acid and PLGA) are the only FDA-approved Polymers that are of great interest. The emulsion-solvent evaporation method is one of the most common [250,279–283]. PLA and PLGA particle synthesis methods are considered and, in this method, Polyvinyl alcohol (PVA) is used as a common stabilizer, with this technique, both large and small particles are obtained, and if 100 nm dialysis membranes are obtained [251–254,284]. The tiny particles with an average size of 702 ± 2 nm is separated and the larger impermeable particles have an average Size of 202 ± 9 nm. Smaller particles are 27 Equal larger in gene Vectors to Cos-7 cells and 4 Equal larger in HEK-293 Cells. The surface load, cell harvest, and DNA release of both groups are approximately Equal [172,173,285–291].

Others have studied the residual role of PVA on cellular uptake as well as on PLGA nano Particles. Removal of PVA during the Preparation Process is very difficult, so some PVA will remain with the Particles, which depends on the amount of initial PVA, the Type of organic Solvent Used in The Emulsion, several other factors. PVA affects Particle Size, Zeta Potential, and Poly disparity index (PDI), nano Particle surface hydrophobicity [119,241–245]. Particles with high residual PVA, although smaller in size, also have low Cellular uptake, which may be due to Further reduced hydrophobicity of the nano Particle Surface [174–176,292–294].

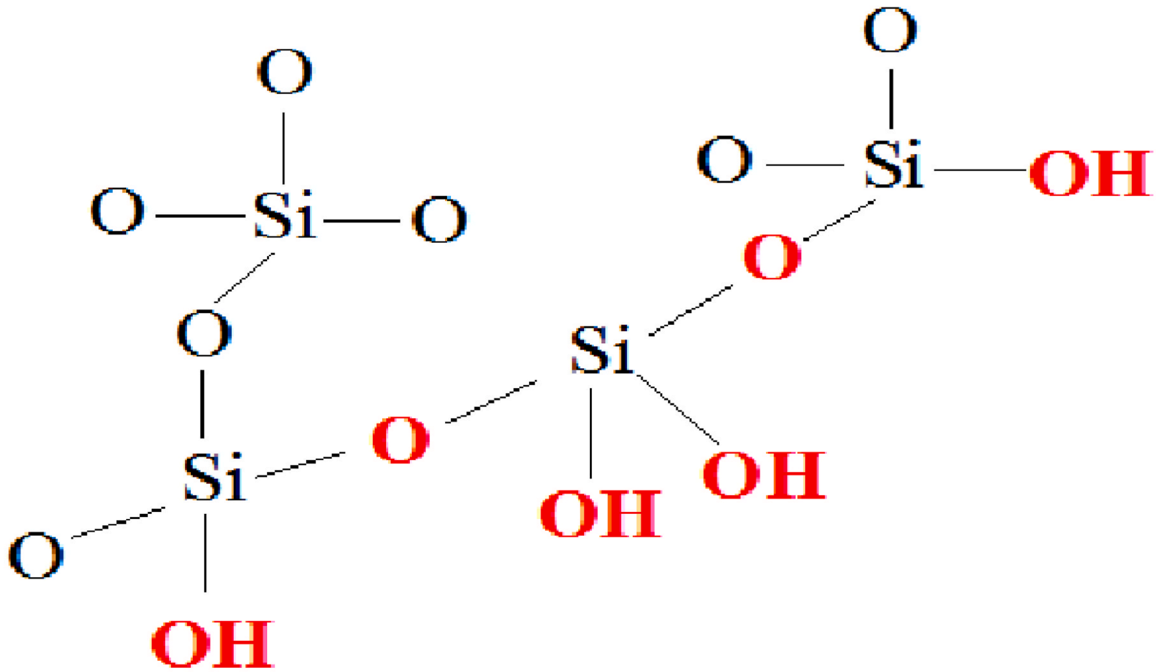
3.5. Silica

The use of silica nanoparticles for gene delivery applications has not received much attention. A series of studies have shown that superficially charged silica particles have the ability to interact with plasma DNA and be used in virus gene Vectors. These particles are produced by changing the level of silica on the market. Compared to PEI particles, no specific toxicity is observed for these particles at the required concentrations [177,178,246–249,295–301]. Fig. 7 shows the structure of silica.

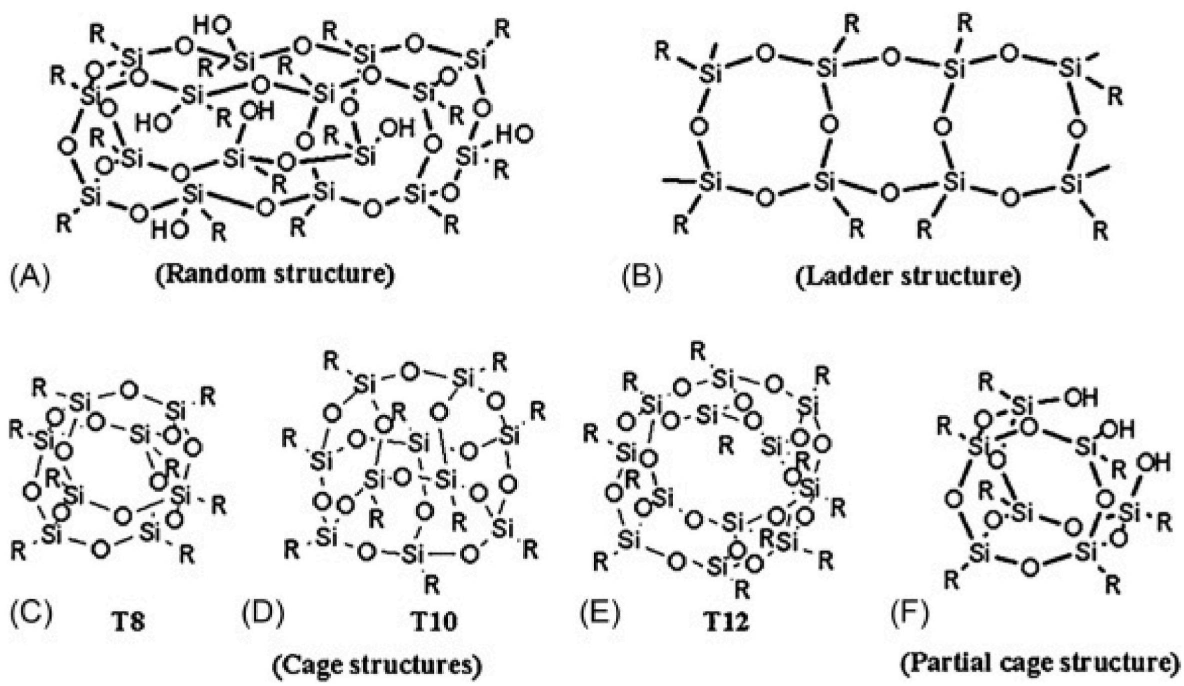
3.6. Block copolymers

Co-polymers are combinations of the cationic and hydrophobic moieties that react spontaneously with DNA polyanion to form micelles.

Kataoka et al. are pioneers in this field of research [179,180]. They have studied various aspects of the construction of these cationic bundles, physicochemical properties, and biological aspects such as cell harvesting and endosomal escape. Fig. 8 shows the synthesis and structure of block copolymers.

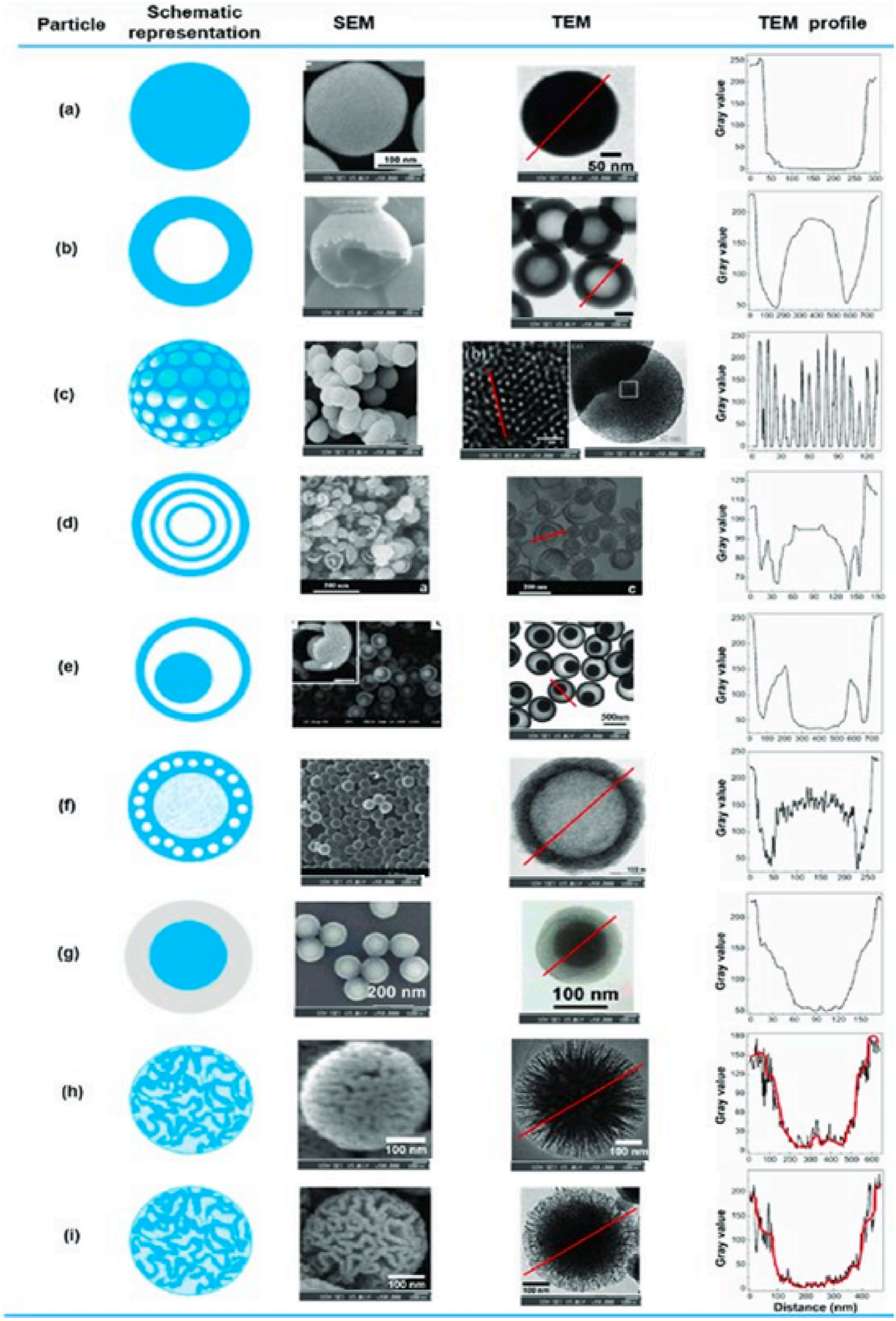


(A)



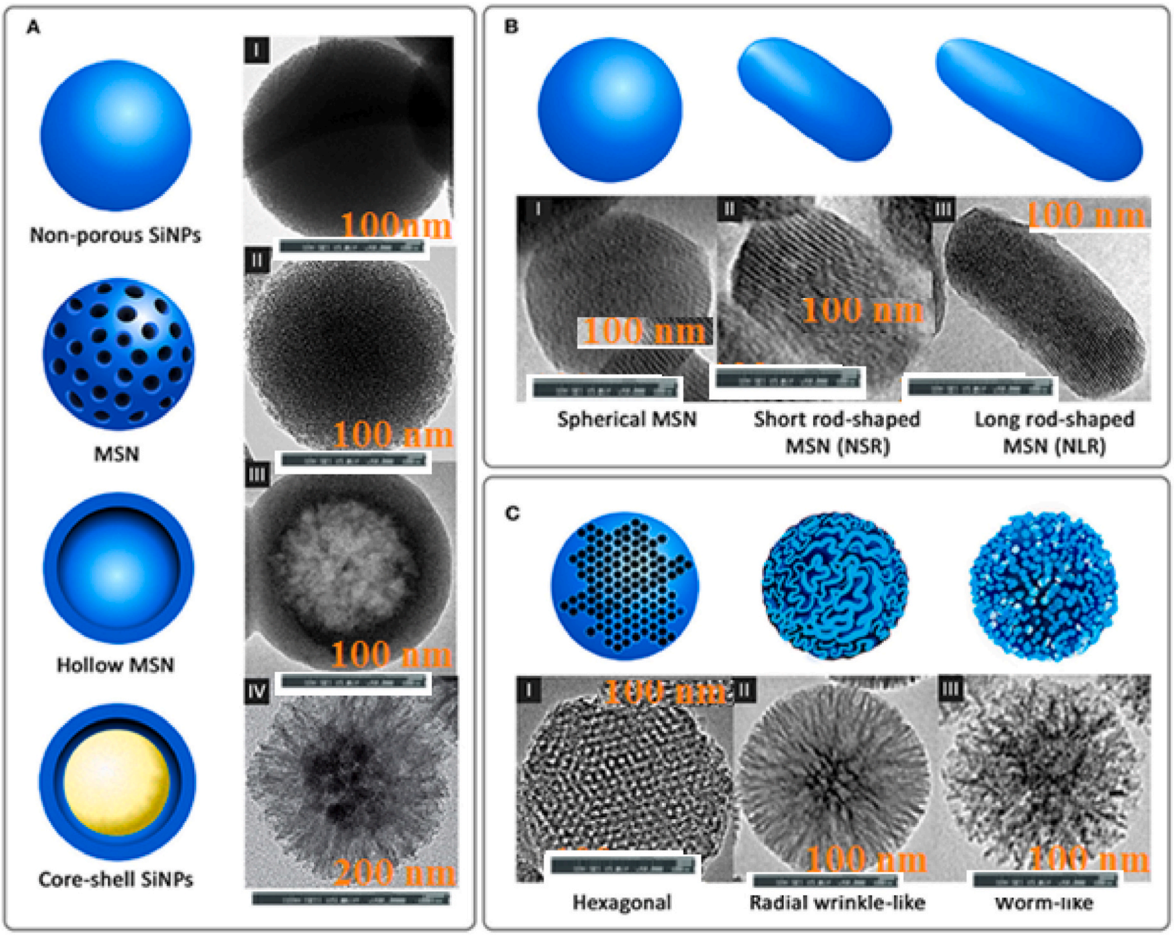
(B)

Fig. 7. Structure of (A) silica (B) silica nanoparticles(C) Silica particles in various morphologies were observed by SEM and TEM images (D) Different forms of silica nanoparticles. Figures reproduced with permission from Refs. [119,292,293].



(C)

Fig. 7. (*continued*).



(D)

Fig. 7. (*continued*).

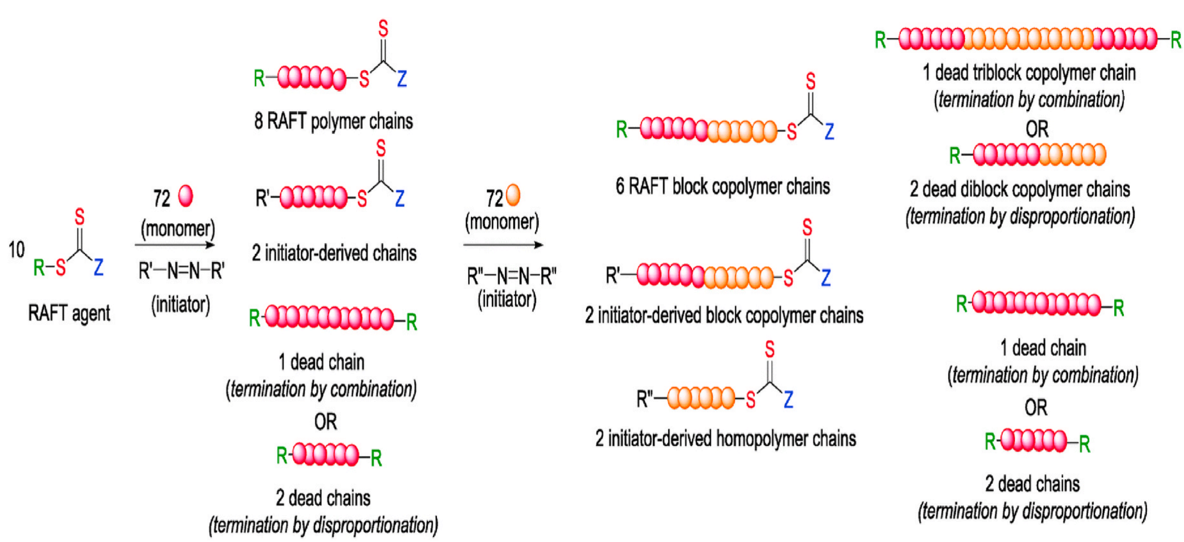


Fig. 8. The Synthesis and structure of Block Copolymers. Figures reproduced with permission from Ref. [195].

3.7. Quantum dots

Burgess et al. showed that plasmid DNA could be covalently attached to Quantum Dots. They encapsulated the quantum points with TOPO = triocytylphosphine oxide and TOP (triocytylphosphine), and then attached the Quantum Dots to the plasmid DNA using a melamine linker containing a disulfide bond. This system showed very low toxicity and high DNA delivery to the cell nucleus. Bhatia et al. also linked the siRNA to Quantum Dots using an endosome escape agent (lipofectamine) and were able to turn off the EGFP (Enhanced green fluorescent protein) gene by up to 29% [177–182,302–308,309–315]. Various applications and advantages of bio functionalized quantum dots have been tabulated in Table 1 [183–186,194].

3.8. Gold nanoparticles

DNA Coated on metal nano particles (for gene transfer) was first used by Particle acceleration Devices (gene guns). In the Initial Studies, DNA was deposited on tungsten nano Particles and these nano Particles were deposited on Large Polypropylene Particles [255–259,316–322]. The device's explosive Pressure is used to Push the Particles, and large Particles Are trapped on the Polycarbonate disk at the End of the machine Tube, while tungsten nano Particles pass through the disk and enter the cells [323–328,329–334]. Sometime later, devices with helium gas Pressure that generated a shock wave were used to Vectors gold nano Particles into tissues. These devices Vectors 4 times more luciferase gene to the skin of mice than Previous series of Devices, but the shock caused by the Pressure of helium gas could cause cell Damage [187,188, 335–341,342,343]. Therefore, later models used helium gas Pressure to launch a bullet. The Pressure from the Bullet stopped the bullet on the disk at the end of the Device Tube, but like Previous models that did not use helium gas pressure, the DNA Particles went to the other side [344–349]. Discs were thrown, causing minimal damage to the cells. In addition, Arc-Discharge guns have been Used as Particle accelerators. These systems contain gold Particles coated on a Mylar film (polyester layer). The electric arc created by the two electrodes accelerates the film on a screen. The Film stops on the screen, but the gold Particles Pass through the screen and reach the cells. In this way, the volume of Particles Vectors by the Device Can Be changed by changing the Voltage, The Density of Au-DNA Particles [189,190,350–354]. Particle bombardment is one of the Primary methods of DNA Vectors that can be used for Immunization applications. Recently, researchers have been thinking about changing the level of gold nano Particles to Vectors their endocytosis to cells.

Rotello et al. [116,119] synthesized monolayer-Protected gold clusters (MMPCs = mixed monolayer protected gold Clusters) by Reacting alkanotiols with 2 nm Gold Nano Particles. Positively Charged Clusters had the highest gene Vectors capacity (68%) and this Group Showed that Gene Vectors capacity was Directly related to the alkyl chain length. Klibanov and colleagues synthesized PEI-conjugated gold nano Particles. The Synthesized Particles were able to Vectors 15 to 6 times more Genes than PEI particles, although the Toxicity of These Particles was Higher than PEI Particles.

Rosi et al. [116,119] attached antisense oligonucleotides with thiol groups to 13 nm gold nano Particles and obtained good Results for EGFP gene silencing. In order to improve the Vectors of oligonucleotides with nano Particles, Efforts have been made to make combinations of nanoparticles and DNA with unstable bonds, which are broken in the cell cytoplasm and the oligonucleotide strand is separated from the nanoparticles. For this purpose, in one of the methods, by increasing the concentration of intracellular glutathione, it is possible to release the oligonucleotide filament that is attached to the particle by binding thiol to the particle [116,119,192,275].

3.9. Carbon nanotubes

The use of carbon nano tubes for gene therapy dates back to recent years. These carbon Structures are highly Insoluble and Covalent and non-Covalent Functionalization Techniques have been Developed to Increase the Solubility of These Structures [260–262,355,356–359]. Civilianization Involves two methods. Oxidation of Carbon nano tubes Under Acidic Conditions Produces Acidic Ends [360–365,366–370]. Non-Covalent Functionalization Typically Involves hydrophobic or Step-by-Step Interactions between Carbon nano tubes and Surfactants, Amino Acids, or nucleotide Strands.

Bianco et al. used carbon nanotubes covalently attached to cationic functional groups to bind DNA. Cell harvesting of these compounds contained DNA in the Hella cell line through endocytosis [263–267, 371–373]. Microscopic results showed that DNA binding to multicolored carbon nanotubes was much stronger than binding DNA to single-walled nanotubes. Special has been done using carbon nanotubes. Dai and colleagues used phospholipid-PEG-functionalized nanotubes to react with thiol-terminated siRNA [268–272]. The terminal amine attached to the nanoparticle reacts with the sulfur group attached to the DNA. These structures have been shown to have excellent siRNA delivery and gene silencing capabilities. In-body tests performed with carbon nanotubes also provide a clear perspective for this method [176–184,374–376].

First described in 1991 [44], carbon nanotubes (CNTs), formed from one or more sheets of graphene shaped in a cylindrical structure, have unique chemical and physical properties. Iijima [44,45] initially discovered multiwalled carbon nanotubes (MWNTs), which have a diameter of 4–30 nm, with two or more graphene cylinders centrically arranged. Two years later Iijima and Ichihashi [45,46] reported on

Table 1
Different approach of bio functionalized QD.

Cognition rate Conjugated to QD	purpose	approach	Benefit	References
Streptavidin	analysis of Biotinylated all human DNA in metaphase chromosomes	Employed in the pathology of medical genetic diagnosis	Ability to resist light and Top sensitivity	[160-**162**]
Angiotensin 2	The Chinese Hamster Ovary (CHO) Cells Is Used For Incubation	–	QD Yield And Photostability Levels are Higher Than The Angiotensin Dye Conjugate	[163]
CdS QD With Carboxyl Group	DNA Molecules	Used in marking DNA Molecules	DNA is quantitatively examined in a more advanced manner, which is associated with stable QD fluorophores	[164]
Adenine and AMP	bacteria is used for incubation	bacteria is marked through mechanisms dependent on Purine	–	[145]
QDs-labeled	–	The syphilis is checked through a new point of care based on QD	Lateral flow test, which is has lower cost, is sensitive, and can be easily used	[170]
Cy3-SSDNA	–	–	to detect solution-based fluorescence, minor DNA changes are necessary	[165]
One End of DNA Hybrid Bound to Streptavidin Conjugated 605QD	–	–	This method in highly efficient in terms of detection potentials, in more appropriate to deal with temperature, and has shorter analysis time as a result of enhanced hybridization efficiency and higher diffusion coefficient	[166]
3-mercaptoropionic acid	–	–	–	[173]

single walled carbon nanotubes (SWNTs), which have a diameter of 0.4–3 nm, and which are composed of a single graphene sheet. While their small size and chemical inertness are attractive properties for delivery of DNA, their hydrophobic nature makes them poorly soluble in aqueous solutions, which places limitations on their application in biological systems. To increase their solubility and dispersion, CNTs can be functionalized through either covalent or non-covalent functionalization [47,48]. The two most common types of covalent functionalization reactions are oxidations and cyclo-additions. While covalent functionalization can improve the biocompatibility and solubility of pristine (chemically unmodified) carbon nanotubes, many of their intrinsic physical properties are either destroyed or weakened [47]. Alternatively, non-covalent functionalization can be carried out by coating the carbon nanotube with amphiphilic molecules, such as sodium dodecyl sulfate [SDS] [49] or proteins [50], which preserves the chemical structure, allowing the CNTs to maintain their unique physical properties [51–54]. MWNTs are generally considered less attractive than SWNTs because of their larger diameter. However, the large diameter is beneficial for the delivery of larger payloads of DNA. Additionally, the toxicity is not based solely on the size of the nanotube, but is also determined by the method of functionalization [47].

Liu et al. [361] covalently functionalized MWNTs with chitosan-folic acid nanoparticles (CS-FA NPs) to deliver plasmid DNA. The group demonstrated that shorter MWNTs have a higher transfection efficiency, but as a consequence, also have a higher cytotoxicity. Yet, when functionalized with CS-FA NPs, the transfection efficiency was increased, while the cytotoxicity decreased [361].

4. Strategies to improve cellular uptake of non-virus carrier systems

Non-virus gene delivery is used to express different genes or to turn them off in animal tissues such as the central nervous system, lungs, liver, etc. Gene delivery with these carriers is often inadequate compared to virus carriers, and gene survival is often poor in virus. Further advances in non-virus Gene Delivery Technology Could Expand Its applications and provide a reliable replacement for viral carriers [117,155,194–196] For in vivo therapies, it is important to target the carrier for a specific cell to prevent effects on other non-target tissues and cells. Active tagging is often done for a series of cells. However, in some cases, depending on the physiological condition, passive targeting of the carrier is also done passively [197–202]. For example, the presence of irregular pores in the endothelial tissue of tumor vessels (Irregular endothelial fenestration) allows the entry of nanoparticles of a certain size or composition. They bring (EPR Effect). To achieve active targeting in-body conditions, the carrier must minimize nonspecific interactions by being Coated with a Polymer Coating Such as PEG and Have specific cell-specific targeting agents. Different ligands are used for this purpose, which depending on the structure and type of target cell can include folate, transferrin, EGF, FGF, aphrodisiacs, Nano bodies, different antibodies [116,119].

5. Target prospects and future

Today, gene delivery research is looking for better carriers that match the patient's characteristics and type of disease. Therefore, the possibility of designing specific carriers for various diseases in the future is high. Most genetic diseases require carriers who can transfer genes of medium and large size. Gene injection is not the only problem with gene delivery. In some cases, the carrier must provide the conditions for the gene to be activated in the target tissue. Therefore, the carrier must have a regulatory device that allows the doctor to turn the gene on and off and change the level of protein therapy. Among these tools, we can mention the promoters with large size and complex shape, but the placement of these promoters in the vector is a major problem. It is very clear That in The Future, polymers will play a key role in advancing gene carriers. By choosing the right molecular weight, they can be optimized for specific applications. By attaching targeting agents to specific tissues or cells or other changes, their biological and physicochemical properties can be improved. Once you are fully aware of the proper carrier structure, it is usually easy to industrialize carrier manufacturing, but it is clear that all of this is only possible for non-virus carriers [116,119].

6. Conclusion

As is evident from this review, many new and innovative approaches to therapeutic gene delivery have emerged in recent years. Nanomaterials appear especially well suited as delivery vectors for small RNA molecules. Given that the vast majority of studies with nanomaterials have been conducted in cell culture systems, it is also clear that additional tests with whole organisms will be necessary before we can expect to see a trend away from the use of viral vectors. Viral vectors are still considered the primary choice for gene delivery, evident by their use in 67% of current clinical trials versus less than 1% of non-viral vectors. For novel nanomaterials, techniques and gene delivery approaches continue to be developed, the key challenge will be to balance transfection efficiency, targeting specificity, particle size, biodegradability, and cytotoxicity, as well as their short- and long-term fates in the environment. This will typically require a mechanism to prevent the removal of vectors by the reticulo-endothelial system (RES). Many nanomaterials can avoid the RES system by shielding their charged groups with PEG, proteins or polysaccharides, as highlighted with a number of examples. Additionally, the smaller the nanoparticles are, the lower the probability of surface neutralization by serum proteins and subsequent RES clearance. Quantum dots are of special interest in this respect. The co-administration of two different nanomaterials to target and deliver gene payloads to create a synergistic effect and the use of theranostic nanoparticles are especially interesting developments that show great promise. Furthermore, the use of standardized assays that can be used to determine the efficacy and fate of a variety of gene delivery vectors would enable head-to-head comparisons. Following such additional research, and detailed characterization of their interaction with the patient host, it is likely that some nanomaterials will be approved for use in humans in the near future, thus expanding the therapeutic repertoire for gene therapy. In the past decades, substantial advances have been achieved in different areas related to gene-based therapy, including the development of new delivery materials, as well as improved potency and stability of nucleic acids. Moreover, advances in genomics have greatly enhanced our understanding of the genetic basis of disease and provided a range of new targets for genetic medicine. Many recently identified disease targets are considered 'undruggable' using small-molecule inhibitors. The potential of exogenous nucleic acids may provide an avenue to translate our knowledge obtained from biomedical research into therapeutics. The broad application of non-viral gene-based therapy will require continued efforts not only to understand the structure–function relationship and the biology behind delivery vectors but also to expand the list of tissues and organs that can be successfully targeted. In addition, non-viral delivery of genome editing systems could facilitate both precise and permanent correction of disease genes. The development of advanced delivery vectors should increase the potential of gene-based therapy to treat a range of different tissues and to silence, correct or introduce specific genes with minimal adverse effects. More than a decade after the initial discovery of RNAi, the promise of RNAi-based drugs seems imminent. Several therapeutic approaches based on siRNA have shown promising results in clinical trials and have illuminated the challenges associated with the application of RNAi in humans. With the recent development of nanoparticle and conjugate formulations, in vivo delivery of siRNAs and/or miRNAs can be achieved with high efficiency and low toxicity, and efficacy has been shown in humans. Although non-viral gene-based therapies have yet to be approved by the FDA, recent progress in the clinical development of RNAi has generated considerable excitement. As discussed, several clinical programmes have reported targeted knockdown in humans following non-viral delivery of siRNAs. One compound, ALN-TTR02, has now transitioned into Phase III clinical trials. More recently, modified siRNAs conjugated to liver targeting ligands have shown efficacy in humans following subcutaneous application, which highlights the possibility of siRNA therapeutics that can be self-administered by patients (see ALN–TTRsc Phase I study results). Taken together, although it will be important to monitor performance of these drugs as they advance into later-stage trials, the efficacy already shown by these formulations in humans, together with the many advances in preclinical development, convinces us that siRNA has a broad and important potential as therapeutics in humans. Whereas the promise of RNAi-based therapies seems to be within reach, there are still certain challenges for successful mRNA- and DNA-based therapies using non-viral vectors. The challenges of stability are greater for mRNA than for siRNA, as the chemical modifications that confer stability on siRNA duplexes can often render mRNAs ineffective. Therapeutic DNA expression additionally requires delivery into the nucleus, which still poses a formidable challenge. Nonetheless, the immense therapeutic potential of these technologies is on the horizon, and the clinical translation of siRNA delivery systems will help to guide us in overcoming these additional barriers.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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