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Nano-materials for Gene Therapy: An Efficient Way in Overcoming Challenges of Gene Delivery

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Abstract

RNA interference (RNAi) is one of the most exciting and revolutionary new approaches to therapies that have attracted considerable amount of attention within the last few decades. Interfering RNAs (iRNA) are a major biological macromolecules that regulate specific gene sequencing, silencing and down regulating. Non coding RNA may lead to the development of a new range of potentially thousands of therapeutics. If efficiently used, iRNA is considered as a potent therapeutic agent for different disease types including viral diseases and cancer. However, the major obstacle that stands in the way of realization of such therapies is the in vivo delivery of RNAi fragments, like small interfering RNAs (siRNAs). The optimal approach to deliver si RNAs would be one to guarantee targeted delivery and high stability, a method that can protect the delivered material from undesirable immune response. In this review, we will shed the light on the use of biocompatible nanoparticles as safe delivery vehicles of genetic material.

Keywords: Nano genetics; Gene therapy; siRNA; mRNA; Non-viral delivery; Liposomes; Polymer nanoparticles; Dendrimers; Gold nanoparticles; Magnetic nanoparticles; Quantum dots

Introduction

Nanotechnology is a field that has been developed some decades ago, since then it has been expanding rapidly; and it has gained the attention of many scientists [1-5]. Nanotechnology applies synthetic chemistry to fabricate Nano scale building blocks, which could be functional on their own, with other materials Recently, novel devices have been fabricated and used from nanomaterial, e.g. new processors, fuel cells, energy storage devices in batteries, LEDs and photo electrochemical cells [6-10]. In addition, bio nanotechnology is a science that made use of biological building blocks to fabricate useful tools at the Nano scale [11-18]. Furthermore, the detection of biomolecules is of immense potential in the direction of molecular sensing in addition to self-assembly [19-21]. Furthermore, other sophisticated structures like animal viruses plus bacteriophages could be assembled at the Nano scale, such bio-nanostructures recognition can lead to the "bottom up" assembly. Furthermore, bio-nanotechnology is the application of biological building blocks intended for the improvement of novel technologies on the Nano scale. Besides, bio-nanotechnology is evidently not restricted to molecular functions; yet, they exhibit a broader capacity. Other functions of bio-nanotechnology include the applications of oligomers, peptide nanotubes, and metal nanowires. Indeed, bio nanotechnology is one of the key technologies of the 21st century that merges material science and biotechnology; it is currently being studied and optimized. This field involves the utilization of biological systems such as cells, cellular components, and proteins, to manufacture efficient nanostructures. Nanotechnology is the new utensil that explores bimolecular structures, functions and properties. Bio nanotechnology made it possible to determine structural elements of cells, molecular recognition and drug delivery [11-13,22-42] (Table 1). In the same vein, gene therapy is a medical intervention that uses genes for the treatment or prevention of disease. If the gene of interest is delivered properly to the desired site, then this strategy would allow the direct insertion of a gene into a specific cell. Gene therapy has gained massive researchers' interest because of its potential to be an alternative for surgery and drug treatments. Gene therapy have been applied to replace a mutated gene that causes disease, knocking out mutated genes, and introducing new genes into cells to help fight a disease [24,43,44]. Even though gene therapy could be a promising treatment option for a number of diseases, its safety is still negotiable. Therefore, different types of biocompatible nanoparticles have been used to deliver genes intended for gene therapy to overcome the disadvantages encountered with the traditional methods used for genetic material delivery. In this review we will shed the light on the types of nanoparticles that have been used to deliver genes intended for gene therapy [45-48]. Moreover, RNA interference (RNAi) is one of the most exciting and revolutionary new approaches to therapies that have attracted considerable amount of attention within the last few decades. It has been found that gene expression may be controlled at the level of messenger RNA via non-coding RNAs. RNAi is an important pathway that leads to explicit gene silencing and down regulating. Non coding RNA may lead to the development of a new range of potentially thousands of therapeutics. If efficiently used, RNAi is considered as a potent therapeutic agent for different disease types including viral diseases and cancer. Plus, microRNA (miRNA) and small interfering RNA (siRNA) may be used as curative agents on their own, as they both adjust gene expression with high specificity [49-61]. A fare example of such an approach is in the treatment of cancer, whereby the siRNA may selectively increase the susceptibility of the cell to the low molecular weight anticancer agent housed in the same delivery system. It is interesting to note that

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Type of Drug Delivery System	Trade Name	Active Ingredient	Breakthrough	Disease	Year of Approval
PEGylated proteins	Adagen®	PEGylated adenosine deaminase	Increased circulation time and reduced immunogenicity	Adenosine deaminase deficiency, Severe combined immunodeficiency disease	FDA 1990
	Cimzia®	PEGylated antibody	Increases hydrodynamic radius, prolongs circulation and retention time, decreases proteolysis, decreases renal excretion	Crohn's disease, rheumatoid arthritis	FDA 2008
Nanocrystals	Emend®	Aprepitant as nanocrystal	Increased bioavailability due to increased dissolution rate.	Emesis, antiemetic	FDA 2003
	Rapamune®	Rapamycin (sirolimus) as nanocrystals formulated in tablets		Immunosuppressant	FDA 2002
Polymer-based nano-formulations	Copaxone®	Glatiramer peptide	Glatiramer is thought to divert as a "decoy" an autoimmune response against myelin	Multiple Sclerosis	FDA 1996/2014
	Genexol®	Paclitaxel	Passive targeting via EPR effect	Metastatic breast cancer, pancreatic cancer (IV)	South Korea 2001
Protein–drug conjugates	Abraxane®	Nanoparticles formed by albumin with conjugated paclitaxel	Passive targeting via EPR effect and may increase endothelial transcytosis	Metastatic breast cancer, non-small-cell lung cancer (IV)	FDA 2005
Surfactant-based nano-formulations	Estrasorb™	Emulsion of estradiol in soybean oil, polysorbate 80, ethanol, and water	Increase drug solubilization	Hormone replacement therapy during menopause	FDA 2003
Metal-based nano-formulations	NanoTherm®	Aminosilane-coated superparamagnetic iron oxide 15 nm nanoparticles	Thermal ablation	Local ablation in glioblastoma, prostate, and pancreatic cancer (intratumoral)	Europe 2013
Virosomes	Gendicine®	Recombinant adenovirus expressing wildtype-p53	Targeted gene therapy	Head and neck squamous cell carcinoma	People's Republic of China 2003
	Rexin-G®	Gene for dominant-negative mutant form of human cycline G1	Targeted gene therapy Specificity achieved by targeting exposed collagen	For all solid tumors	Philippines 2007

Table 1: Few examples of FDA approved polymer nanoparticles based therapies.

such approaches may also benefit from appropriate sequential release of two types of agent, although this consideration is in its infancy and is currently secondary to the considerable engineering challenges associated with the development of such nanostructured systems (Figure 1 and Table 1).

Challenges of delivery of therapeutic siRNA

SiRNA have great potential to be a leading therapeutic tool for several diseases. However, the major obstacle that stands in the way of realization of such therapies is the *in vivo* delivery of RNAi molecules, like the small interfering RNAs (siRNAs). Many intracellular and extracellular obstacles still need to be conquered in order to benefit from the full aptitude of this technology. The molecules are too impermeable and too metabolically labile to be delivered alone, hence it is essential to develop vectors with which these molecules may be both protected and facilitated in reaching the target site.

First of all, siRNA stability is highly negotiable, due to the extracellular degradation by enzymes located in serum and tissues, resulting in a short life time of the bare siRNAs in serum that can go up to one hour [34]. Hence, the targeting of therapeutic siRNAs is extremely challenging, as the siRNA faces many barriers before reaching their target cells to act on the gene silencing. Additionally, when the siRNAs are in the cell cytoplasm, they become susceptible to deterioration as a result of their contact with the intracellular RNAses. Secondly, another issue that should be taken into account when dealing with therapeutic siRNA is the off target silencing. Sometimes the specific silencing may suppress other genes than the ones of interest. Resulting in major undesirable mutations of gene expression, therefore, analytical bioinformatics methods are recommended. At this stage, siRNA design promises to considerably minimize and ultimately eliminate off-target silencing. Also, siRNAs may trigger immune responses by activating interferon responses resulting in cell death. Immune response is different from one cell to another, which makes it hard to predict the *in vivo* behavior without running *in vitro* experiments first. An optimal delivery strategy for siRNAs would be one that guarantees targeted delivery and high stability, a method that can protect the delivered material from undesirable immune response. In this project we will focus on the synthesis of a bio compatible delivery system that can specifically target the delivery of siRNAs, protect it from elimination and increasing the chances of the medical applications of siRNAs therapies. A trend that is pertinent to these discussions is the use of Nano composite systems, whereby more than one vector (a complexing molecule, a lipid or a synthetic polymer) may be combined into a Nano particulate system in order to elicit more than one advantage in delivery.

Therapeutic siRNA

The field of RNA interference (RNAi) started to gain much interest, since it was uncovered by Fire and Mello, around two decades ago. The standard understanding of the gene regulation has been transformed after the functional studies performed on C. elegans, where it has been found that double stranded RNAs were the reason of the gene silencing in C. elegans [62-65]. Subsequently, siRNAs have been found in plants and showed to direct sequence-dependent endo nucleolytic cleavage of the mRNAs that they regulate in mammalian cells [66-68]. Moreover, some years later Elbashir. had effectively utilized synthetic siRNAs for gene silencing and they were able to verify the basic siRNA structure, offering the basics for optimizing RNAi applications [69,70]. Ever since, selective silencing of genes became possible. Selective gene silencing can be achieved by controlling the endogenous RNAi pathway with synthetic assemblies. This technique

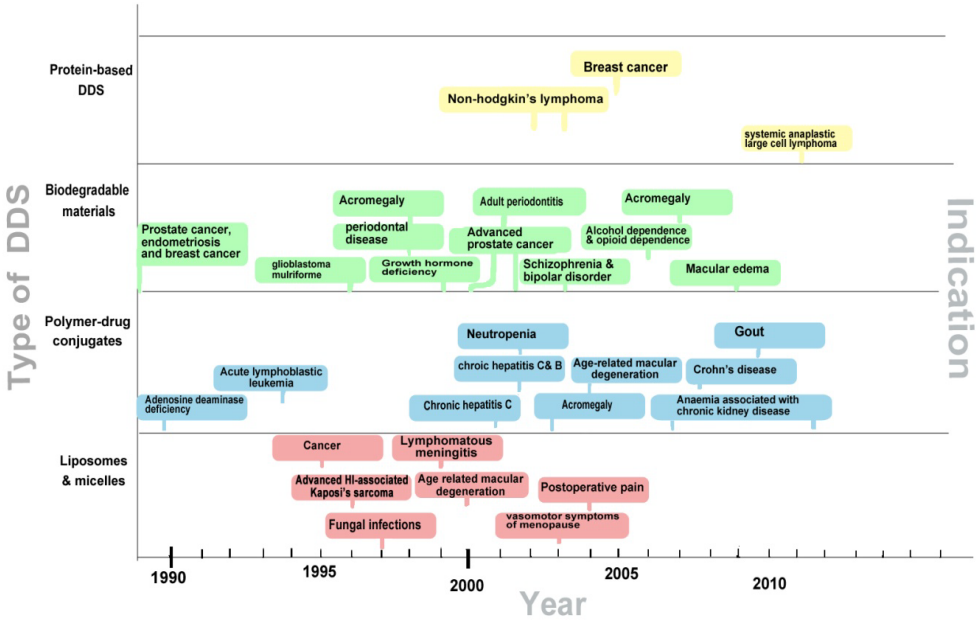

Figure 1: Different drug delivery systems and their use in therapeutics.

is becoming widely popular for the genetic functional studies, where it has resulted in promising therapeutic aptitude. However, the realization of using siRNAs as therapeutic agents is hindered by many challenges. siRNA Stability in serum is highly negotiable, siRNAs are liable to degradation by serum and tissue enzymes; resulting in a very short half-life ranging from several minutes to an hour [68,69]. In addition, microarray studies have shown that off-target silencing can be a result of the siRNA therapy, which leads to the suppression of non-interest genes. Off-target silencing may cause dangerous mutations of gene expression and undesirable cellular transformations. It has been shown that most off-target silencing is a result of homology with six to seven nucleotides in the "seed region" of the siRNA sequence [71-74]. Also the use of siRNAs as therapeutics has been limited due to the activation of unexpected immune responses, that can lead to cell death *in vitro* [75,76]. The immune responses differ from one cell to another. Therefore, the *in vivo* immune reactions cannot be anticipated based on the *in vitro* work. The *in vivo* delivery of genetic is the major challenge that faces scientist when developing siRNA based therapies [76,77]. Virus-based delivery systems have been considered as an efficient mean of siRNA delivery. However, it could not be taken any further due to its fatal side effects; virus delivery can stimulate mutations, and trigger immunogenic and inflammatory responses [78]. Hence, alternative no viral delivery systems have been developed to replace the viral delivery of siRNA. Other non-viral delivery systems include direct chemical modification of siRNA, nanoparticles, and targeting moieties.

Nanoparticles used in gene delivery

Polymer nanoparticles: Polymer nanoparticles (PNPs) deliver genes or therapeutic proteins including drugs which can either be dissolved or encapsulated within them forming a nanoparticle and a Nano capsule respectively. PNPs can also deliver proteins to the targeted cells by entrapping them within its structure forming a Nano sphere. The delivered therapeutic proteins or drugs act by altering defective proteins or genes in the patient's cells. The size of

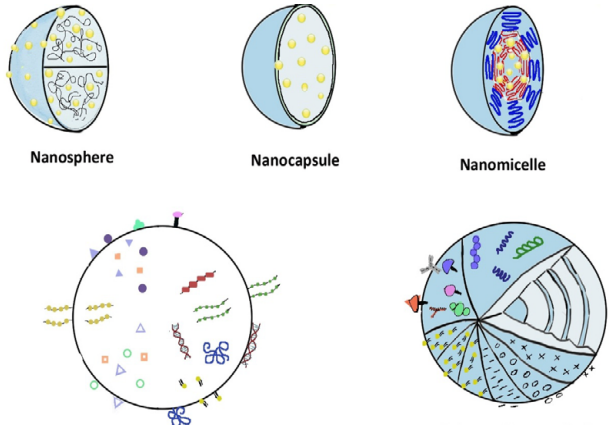

Figure 2: The structure of polymer nanoparticles.

the polymer nanoparticle could be tuned to enable these drugs and therapeutic protein to fit in. PNPs, like all nanoparticles are capable of regaining their size once inside the cell through the physiological change in pH. Figure 2 below represents the structure of polymer nanoparticles. PNPs have been utilized in drug delivery, where they have shown high biocompatibility and high encapsulation capacity. They are great candidates for gene delivery, because they are highly stable and they offer controlled release of active ingredients. Also, PNPs can be used for targeted delivery by surface modification, and they allow the delivery of combined active materials. PNPs are synthesized from non-toxic biodegradable, biocompatible polymers like, Chitosan, cyclodextrin, polyethylene mine (PEI), poly(lactic-co-glycolic) acid (PLGA), and dendrimers [69,75]. These polymers can be used on their own to synthesize (PNPs) also; they could be combined together to get better properties of nanoparticles (Figure 2). PNPs

technology has revolutionized the field of biology and health services. It has facilitated the development of new treatment methods with improved efficacy for treating diseases which had once been viewed as incurable like genetic, immunological and neural disorders [36]. In some cases, the delivered genes act by enhancing the functions of the cells. Polymer nanoparticles are used to overcome the various challenges that have been encountered in using gene therapy [79]. Some genes have relatively long base sequences which make it difficult for them to be delivered to the desired sites. To fit into the target cell, the DNA must be condensed into the Nano structures, to permit their internalization within the cells80. Moreover, the nucleases in the target cells may also degrade the DNA being delivered. And because the gene and the carrier are usually conjugated, their separation at the point of delivery is sometimes difficult. In some cases, gene silencing may also arise as the target cells may act against the delivered genes. Putnam et al. have demonstrated that using polycations such as polyline can overcome the DNA size barrier as it "can condense DNA into toroidal nanostructures" to sizes less than 150 nm which can internalized within the cell. Researchers have also identified various ways in overcoming the challenge of separation of the DNA from the carrier. Using nanoparticles to conjugate the DNA, researchers have developed an effective way to ensure that the genes are delivered to the targeted cells (Figure 3) Mohammedi have synthesized DNA-Chitosan nanoparticles to deliver DNA to the Lung Epithelial cells [80,81]. Also, in 2014 Tang have utilized chitosan based (PNPs) Trimethylated chitosan has been synthesized as gene delivery systems, TMC-g-PCL/DNA polyplexes have shown high uptake efficiency than PEI/DNA polyplexes [22,82]. Plus, Das et al. have utilized PEI based nanoparticles to deliver siRNA to STAT3 in lung cancer, *in vitro* and *in vivo* [81]. Other research groups have also synthesized chitosan as the main targeting nanoparticles for siRNA delivery to treat different diseases like, lung cancer, ovarian cancer, pancreatic cancer and hepatocellular carcinoma [22,81-90]. In 2015, Bishop have utilized polymer coated gold nanoparticles for DNA and siRNA delivery, where this type of inorganic nanoparticles have shown good results in gene silencing [91]. Colombo et al. have synthesized hybrid lipid-polymer nanoparticles for siRNA delivering [92]. While, other up to date studies have shown the improved cancer treatments obtained with co delivery [90-96] (Figure 4).

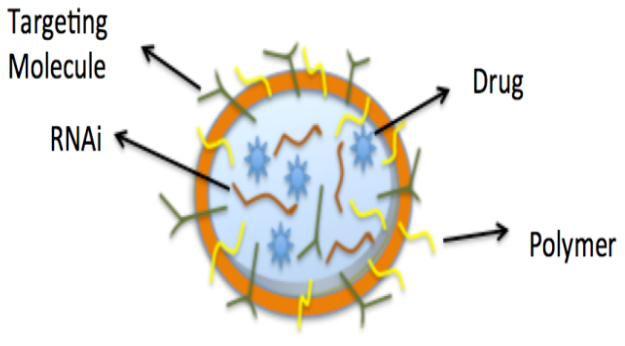

Figure 3: Schematic illustration of therapeutic siRNA loaded nanoparticle.

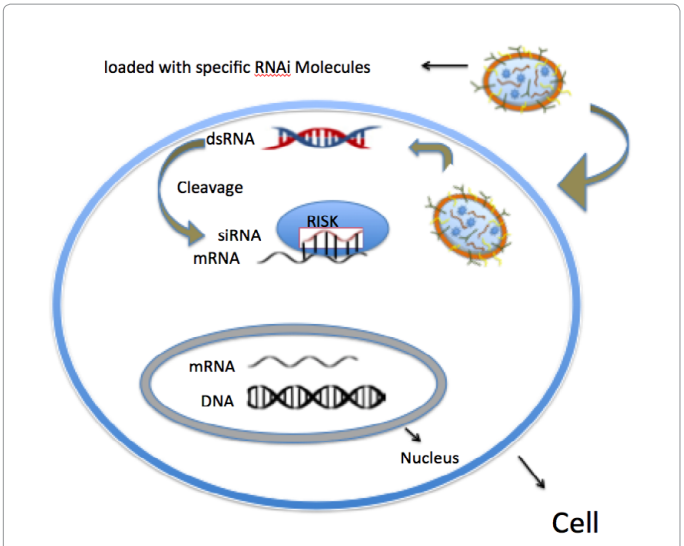

Figure 4: Illustration of the process of RNAi delivery using nanoparticles.

Dendrimers for gene delivery: Dendrimers are 1-10 nm, three-dimensional globular synthetic macromolecules. Dendrimers are highly branched and characterized by monodispersity [97-101]. Synthesis of dendrimers was first discovered by two groups: Buhleier who focused on the construction of low molecular weight amines, and Tomalia who developed the divergent method tosynthesisedendrimers [102]. Dendrimers architecture consists of the core, branches and many terminal functional groups. The core is an atom or a molecule at the centre of the dendrimers with at least two identical chemical functions. From the core, branches with repeated units originate and spread, by having at least one branch junction, to form generations. These branches end with terminal functional groups at the surface of the dendrimers, which dictate the properties of the dendrimer macromolecule [97] (Figure 5). The most well studied dendrimer is polyamidoamine (PAMAM), which is characterized by high solubility and reactivity due to the presence of empty internal cavities and numerous functional groups at its periphery [103]. The properties of dendrimers, such as monodispersity, well-defined structure and the extensive quantity of surface functional groups, made them valuable tools to be used in gene delivery [104] (Figure 5).

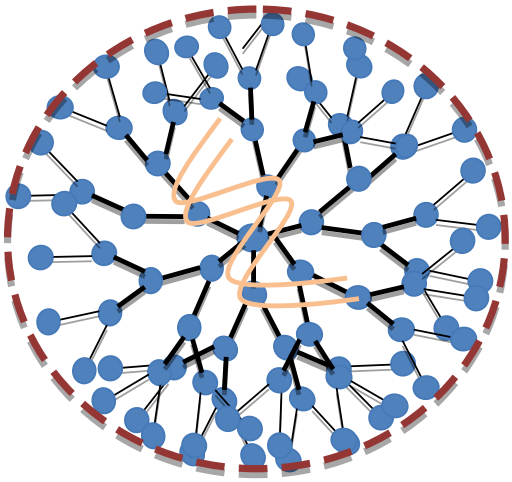

Figure 5: Structure of Dendrimers.

Divergent and convergent methods of synthesis: Dendrimers are usually prepared using the divergent or the convergent method of synthesis. The divergent method of synthesis, developed by Tomalia, is a stepwise method starting from the core of the dendrimer and growing outwards towards the periphery. The multifunctional core reacts with the reactive group of monomer molecules giving the first dendrimer generation, This periphery molecule is then activated to react with the next set of monomers building up the dendrimer layer by layer (Figure 2) [105,106]. The multiplicity of the core depends on the number of molecules added to the core and is equal to the number of branches branching out of the core [107]. Polyamidoamine (PAMAM) dendrimers are prepared using this method of synthesis [105]. Problems that occur with the divergent method result from the side reactions and incomplete reactions of the end groups. These can be overcome by adding large excess quantities of the reagent to force the reactions to completion [106]. On the other hand, an advantage of the divergent methodliesin the ability to change the end groups of the periphery, which modifies the surface and properties of the dendrimer [105]. In contrast, structural uniformity is difficult to maintain with the divergent approach as the number of reactions increases exponentially with each step [108,109]. Hire demonstrated a divergent approach to synthesis "aliphatic ester dendrimersby anhydride coupling" using only a small excess of the reagent and only extraction and precipitation as a purification method. The convergent method of synthesis, developed by Hawker and Fréchet, is a stepwise method is initiated from the terminal groups and builds up towards the interior going to the core (Figure 4) [106]. This approach was developed to overcome weaknesses, such as the low reactivity, of the divergent method [105]. An advantage of the convergent method is the faster reaction rate due to the minimal reactive sites available during the proliferation process. Another advantage is an advanced purification process due to the large "molecular difference" between the end product and the reactant, which leads to an improved separation during purification [105]. On the other hand, the convergent method displays a disadvantage in the inability to produce high generations due to steric hindrance in the reaction between the dendrons and the core [110]. "Hypercores" and "branched monomers" is an advanced method that accelerates the rate of dendrimer synthesis and involves the pre-assembly of oligomeric species that are linked together to generate dendrimers. The "double exponential" is another approach, which begin with a single starting material to prepare monomers for both the divergent and convergent methods. These two products then react to give an "orthogonally protected trimer", which acts as the repeating unit in this growth. Moreover, lego chemistry method was developed by Tomalia and Svenson to simplify the duration and cost of dendrimer synthesis. It involves the preparation of phosphorus dendrimers by employing of highly functionalized cores and branched monomers. This allows the multiplication of terminal functional groups from 48 to 250 in just one step [97,111]. The click chemistry method was developed to produce higher purity and yield compared to the divergent method. It involves the spontaneous synthesis of two monomeric units with complimentary functions avoiding the use of activating agents and reducing the duration of synthesis. This method was successful in the production of triazoledendrimers [111,112] (Figure 6).

Dendrimers for gene delivery: Dendrimers are a great tool for gene delivery as they can interact with DNA, RNA and antisense oligonucleotide through electrostatic interaction to form complexes that condense the nucleic acid [113]. Hyperbranched dendrimers are more suitable to be used as gene delivery tools than more structured dendrimers as their flexibility allows them to form more compact

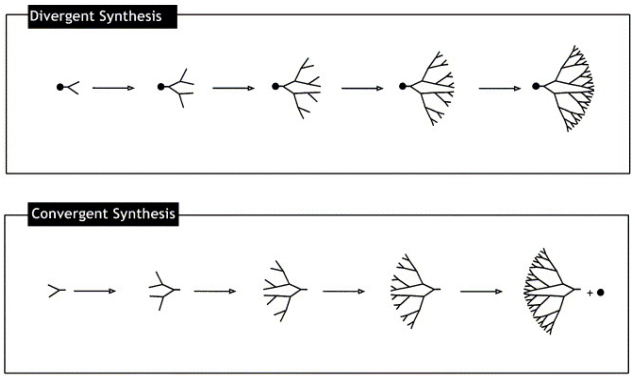

Figure 6: Methods of Synthesis of Dendrimers. a. Divergent synthesis begins from the core and builds up to the periphery. b. Convergent synthesis starts from the branches and builds up inwards toward the core [112].

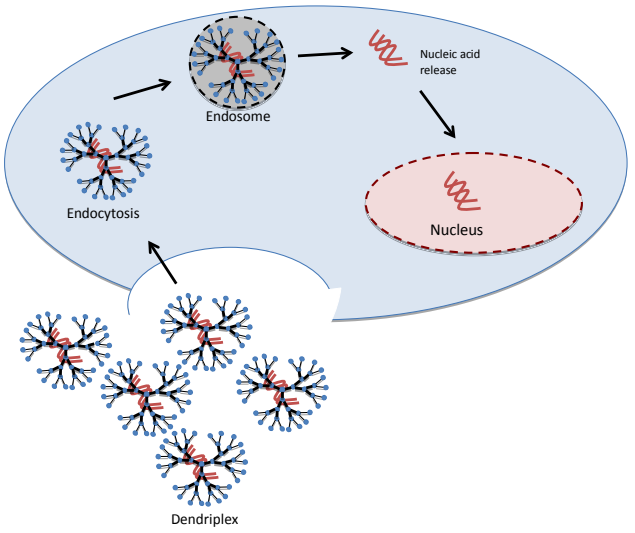

Figure 7: Dendrimers-mediated gene delivery.

complexes with DNA [114]. Under specific physiological and chemical conditions, dendrimes form polycations, which are able to bind to the negatively charged nucleic acid. As this dendrimer-nucliec acid complex needs to cross the epithelia to get to its target, it is required to have a positive net charge to enable the cellular uptake of the complex through its binding to the negatively charged cell membrane. Generally, high generation dendrimers are more toxic than low generation dendrimers. Therefore, the major factors affecting the permeability of the dendrimer-gene complex are the surface charge, concentration, generation time and surface modifications [104] (Figure 7). The positively charged dendrimer-nucleic acid complex (dendriplex) binds to the negatively charged cell membrane and is taken up by endocytosis forming an endosome. The endosome destabilises due to the sponge effect of the dendrimer, releasing the nucleic acid to the cytoplasm. Nucleic acid is then taken up by the nucleus where it is replicated.

Polyamidoamine (PAMAM): The six-generation PAMAM dendrimers are widely used dendrimers as vectors for gene delivery. PAMAM structure shows a high density of amines in the periphery, which enables the condensation of nucleic acid. On the other hand, the inner amines enable efficient endosomal escape through the proton sponge

effect [115], which is the resistance of cationic carriers that comprise a secondary or tertiary amine group to endosomal acidification through the absorption of protons. These cationic dendrimers absorb protons to below the physiological pH, which in turn delays the lysosomal fusion to the endosome. This prevents the degradation of nucleic acid and allows the accumulation of counter-ions like Cl- in the endosome leading to the endosome rupture and the release of its content into the cytoplasm due to vesicular swelling [116]. According to Braun [117], dendrimers with higher generations show better gene transfer than those with lower generations [117]. However, higher generations of the PAMAM dendrimers show higher toxicity due to the increased non-biodegradability. Nevertheless, it has been shown that six generations PAMAM dendrimers are the most efficient for gene delivery [104]. Modified PAMAM dendrimers are the most commonly used for the delivery of DNA and siRNA. A study by Tang showed that PEG (polyethyl glycol) conjugated PAMAM dendrimers has dramatically decreased cytotoxicities than non-PEG conjugated PMAM dendrimers. It also demonstrated that PEG-conjugated dendrimers protected siRNA from being digested and gave high transfection efficiency [118]. Although PEGylated, hyper branched PAMAM dendrimers showed a significant reduction in cytotoxicity, it also demonstrated a significant decrease in gene delivery efficiency compared to unmodified hyper branched PAMAM dendrimers. However, a mixed system that is composed of 30% modified (PEGylated) hyper branched PAMAM and 70% unmodified hyper branched PAMAM dendrimers improves gene delivery efficiency significantly while maintaining low cytotoxicity [119]. Acetylation and internal quaternization of PAMAM dendrimers is another modification that has been shown to decrease cytotoxicity in addition to genotoxicity, formation of micronuclei, of the dendrimers. This modification resulted in a neutral surface dendrimer with cationic charges inside the dendrimer, however the conformed dendrimers resulted in the formation of condensed spherical SiRNA polyplex, which protects the nucleic acids from degredation and improved their cellular internalization [120].

Polypropylenimine (PPI): Polypropylenimine (PPI) dendrimers are ideally suited for DNA binding and gene delivery, as they are comprised of 100% protonable nitrogen [121]. Schatzlien showed that gene delivery using PPI dendrimers demonstrated preferential expression of genes in liver, as opposed to other organs, which facilitate the specific use of it, for example in targeted cancer therapy [122]. Moreover, it has been shown that modification of PPI dendrimers would provide more effective intracellular delivery of the gene. Kim has shown that conjugation of PPI with arginine resulted in low toxicity and high transfection efficiency [123]. Furthermore, PPI dendrimers has also been used for siRNA delivery as shown by Tartula. Where PPI dendrimers were condensed with siRNA to form particles, that are caged with dithiol, and coated with PEG exhibited reduced genotoxicity, increased siRNA cellular bioavailability and stability in plasma, which in turn provided efficient gene silencing [120,124]. Russ have shown that although the generation 2 (G2) plasmid DNA-PPI complex (polyplex) demonstrated lower cytotoxicity than the G3 polyplexes,polyplexes of G2 exhibited lower transfection efficiency than the G3 ones. Moreover, grafting the G2 and G3 PPI polyplex with oligoethyleninime(OIE) showed enhanced transfection efficiency compared to the unmodified counterpart [125].

Polyethylenimine (PEI): PEI dendrimers are water-soluble polymers that can interact with the DNA, because its positively charged, and protect DNA from degradation; which makes them a great delivery tool for siRNA and DNA [126], however some studies show that PEI is less effective in siRNA delivery due to the reduced electrostatic interaction resulted by the short length of siRNA. PEI exerts the proton sponge effect to release the nucleic acid into the cytoplasm [127]. In 2008, Intra and Salem studied the gene transfection efficiency of PEI-pDNA *in vivo* and *in vitro* and showed that the branched PEI-pDNA structures displayed greater efficiency *in vitro*, whereas linear PEI-pDNA structures have shown a greater efficiency *in vivo* when injected intraperitoneally. Moreover, differences in PEI nitrogen: pDNA phosphate ratios also had an impact on transfection efficiency [126].

Other types of dendrimers: Glyodendrimers, which are dendrimers that are incorporated with carbohydrates, has shown a great potential in targeted gene delivery [104]. Cyclodextrins are cyclic oligosaccharides that are composed of a hydrophilic exterior and hydrophobic interior. Wada showed that mannose-conjugated α-cyclodextrins PAMAMs demonstrated high transfection efficiency in mouses' kidney 12-hourpost intravenous administration, compared to the unmodified dendrimers and α-cyclodextrins. Efficient gene delivery in addition to low toxicitymakes it an ideal non-viral vector [128]. Moreover, Arima reviewed "sugar-appended" dendrimers and demonstrated that mannosylatedα-cyclodextrinsdendrimers exhibit high transfection efficiency possibly due to the increased protection of plasmid DNA from methylation compared to unmodified dendrimers. Additionaly increased gene activity displayed by galactosylatedα-cyclodextrinsdendrimers may be due to intracellular trafficking and/or the stability of plasmid DNA [129]. Futhermore, peptide dendrimers, which are dendrimers that contain peptide bonds, have also been reported for gene delivery. A study by Luo synthesised poly(L-lysine) dendrimers as vectors for gene transfection *in vitro* and showed that the dendrimer-pDNA complex protected pDNA from degradation by nucleases with an efficiency that is stronger than the commercially available branched PEI. When compared to PEI, generation 5 (G5) of the dendrimers displayed similar transfection efficiency but lower toxicity to cultured cells [130]. Moreover, Arginine functionalized peptide dendrimers, synthesized using click chemistry, also showed high transfection efficiency *in vitro* independent of serum compared to PEI, specially generation dendrimers which displayed high transfection efficiency *in vitro* and *in vivo* making it ideal for gene delivery [128]. Table 2 below lists some of the approved dendrimers based therapies, used in genetic material delivery (Table 2).

Liposomes for gene delivery: Liposomes are small vesicle-like structures that are formed by self-assembly through lipids energetic interactions. Each phospholipid consists of a hydrophobic hydrocarbon tails, hydrophilic head group and a linker bond that joins the hydrophilic head and hydrophobic tail [131-133]. Liposomes possess properties such as reduced toxicity, safe preparation and reduced risk of immunological rejection, which enable its use for non-viral gene delivery [134]. Cationic lipids, which attain amine groups in the polar head, are more commonly used for gene delivery whereas anionic liposomes uses are constricted to other therapeutic macromolecules, because the positive charge of the liposomes binds to the negatively charged nucleic acids much easier [132]. The use of cationic liposomes for gene delivery is advantageous as it is biodegradable after administration *in vivo*, biocompatible and its surface can be diversely modified when using pegylated lipids [135]. The lipoplex formation is mainly enhanced by electrostatic reactions linking the DNA phosphate backbone and the positively charged polar head group of the liposome [136]. The size of the lipoplexes is determined by the ratio of cationic lipid-to-DNA charge during preparation. Relatively neutral charge ratios with slight excessive positive charges result in the formation of large aggregates, whereas high positive or negative charges results in the formation of relatively small aggregates

Dendrimer	Commercially Available	Applications
PAMAM	STARBURST® dendrimers (Sigma Aldrich)	• *In vitro* transfection of liver (HepG2) and colon (CT26) cells
		• *In vivo* gene delivery
		• *In vitro* transfection into mesenchymal stem cells
		• Formation of stable PAMAM-transferrin conjugate to form stable dendriplexes with plasmid DNA and improved gene delivery to HeLa, HepG2 and CT26 cell lines.
		• Delivery of sticky siRNA *in vivo* and in vitro to prostate cancer model using triethanolamine (TEA)-core PAMAM dendrimer of generation five.
PPI	Astramol (Dutch State Mines (DSM) Netherlands and Aldrich Chemical Company)	• siRNA delivery
		• Successful gene expression in liver rather than other organs
		• Accumulation in tumor tissue and induction of tumor- specific gene expression
		• PPI-collagen conjugates used as a scaffold for corneal tissue engineering.
		• Generation 4 and 5 PPI dendrimers to knockdown mRNA in A549 human lung cancer cells
PEI		• Gold standard for plasmid DNA delivery
		• Delivery of short hairpin RNA (shRNA) to retinal ganglion cells
		• Pulmonary gene delivery *in vivo*
Glycodendrimers		• *In vivo* transfection of mannose-conjugated α-cyclodextrins in mouse kidney
PLL		• *In vivo* transfection of generation 6 PLL-plasmid injected intravenously in mice
		• *In vitro* transfection in different cells
		• Effective knockdown of GAPDH in rat hepatoma (H4IIEC3) cells with low cytotoxicity.
Carbosilane		• 2G-NN16 and 2G-03NN24 carbosilanedendrimers in gene therapy of HIV infection

Table 2: Commercially available dendrimers used as vectors for gene therapy.

[136,137]. The most commonly used cationic liposomes utilized for the delivery of nucleic acids include DOTMA, DOTAP and DC-Chol. First reported liposomes by Felgner was N-(1-(2,3-dioleyloxy)propyl)-N,N,N trimethyl ammonium chloride) (DOTMA), which consists of a monovalent quaternarey amine head connected to two hydrocarbon tail via an ether group [134]. Subsequently, [1,2-bis(oleoyloxy)-3-(trime- thylammonio)propane] (DOTAP), which consist of a quaternary amine group linked to a glycerol backbone and two oleoyl chains, was repored by Leventis and Silvius. The ester bonds in DOTAP provides biodegradability and reduce toxicity because ester bonds are hydrolysable [132]. Furthermore, the cholesterol based liposome, 3β[N-(N',N'-dimethylami-noethane)-carbamoyl]cholesterol(DC-Chol), has been synthesized using cholesterol chains as hydrophobic tails as it provides stability and biocompatibility [132,138]. More recently, new cationic liposome , DODAG (N′,N′-dioctadecyl-N-4,8-diaza-10-aminodecanoylglycine amide), has been reported for efficient transfection of pDNA to multiple cell lines including Hela cells [139]. Transfection efficiency of liposomes is affected by the net charge of the lipoplex in addition to the size of the lipoplex. It has been shown that relatively large liposomes (0.4-1.4 um) has a greater transfection efficiency than smaller liposomes [136-140]. Moreover, it has been proven that PEG modification of liposomes increases the stability of liposomes in blood and improves its pharmacokinetics and transfection efficiency [141]. This prolonged circulation time was shown to be influenced by the length of the acyl chain of the PEG lipid as shown by 142 that explained that longer acyl chains demonstrates a higher transfection efficiency than shorter acyl chains or unsaturated chains. PEG coating also lowers protein binding to the surface of the liposomes, which reduces uptake by macrophages. Higher blood circulation is also influenced by the molar percentage of PEG lipids, however higher percentage also hamper cellular uptake and cytoplasmic deposition of siRNA [142,143].

Magnetic nanoparticles: Paramagnetic nanoparticles have been used as drug carriers. Their accumulation is guided in target tissues using strong magnetic fields, and they has successfully used in cancer treatment. Similarly, the same technique has been applied to gene vectors; a high throughput magnetofecion was able to offer a new tool for gene therapy that overcomes the drawbacks of *in vivo* gene therapy. Magnetofecion (Figure 8) has improved the efficacy of conventional transfection methods *in vitro* and *in vivo*. The first application of magnetic nanoparticles in gene therapy was reported by Scherer et al. [143]. They have associated gene vectors with superparamagnetic iron oxide nanoparticles. *In vitro* and *in vivo* experiments have shown that gene delivery and targeting was enhanced by the magnetic force within the iron oxide nanoparticles. Moreover, in 2005 Morishita have incorporated magnetic nanoparticles into unique vectors called "HVJ-E (hem agglutinating virus of Japan-envelope)", where the magnetic nanoparticles have improved the transfection efficiency in *in vitro* studies [141]. Advances in technology have recognized magnetic nanoparticles as therapeutically reliable delivery systems, that is able to both enhance transfection of cargo as well as allow for target specific delivery through external application of a magnetic gradient onto the desired area [144]. The term "magnetofection" coined by Scherer refers to this method of magnet-assist gene delivery [145,146]. The gene is joined to a magnetic particle or transporter, consisting of an iron-oxide encapsulated within a polymer or metallic shell [143]. Alternatively, the particle can be dispersed in a polymer matrix. The shell or matrix may then be functionalized through attaching amines, biotin, streptavidin or antibodies in order to achieve maximum efficiency. Nanoparticles used for *in vitro* reactions are layered with polyethylene mine (PEI), which is able to adhere to DNA by means of electromagnetic interaction. In addition, the highly positive charge of PEI also further supports DNA transfer into cells as well as promotes dissociation of DNA complexes from endosomes through a proton sponge effect that ruptures the endosome [147,148] which relies on coating iron oxide nanoparticles with cationic polymers and particularly poly-ethylenimine (PEI) [149]. The few studies mentioned above represent most of the work done combining gene therapy and magnetic nanoparticles. Although the previous studies have shown an enhancement in transfection, with such promising results, not many investigations have been reported recently (Figure 8).

Gold nanoparticles: Gold nanoparticles (AuNPs), are known to be

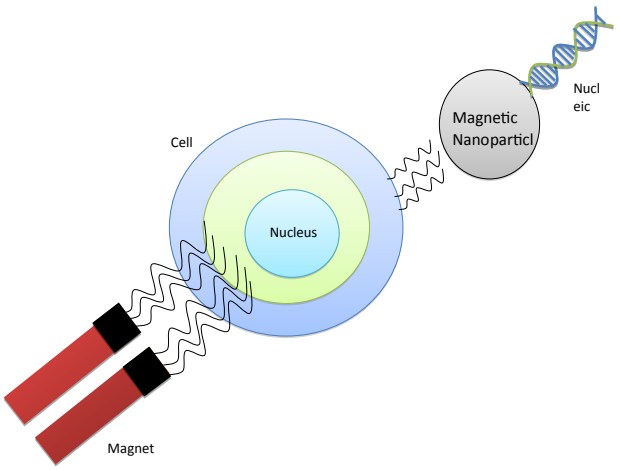

Figure 8: Schematic illustration of the process of Magnetofecion. The delivery of genetic material using magnetic nanoparticles.

appropriate gene delivery vehicles. The optical and physiocochemical properties that allow for easy transfection into cells as well as their unique biocompatibility that make them non-toxic. Moreover, AuNPs can be easily modified and custom made for optimum delivery and specificity. Several issues must be taken into consideration for successful gene delivery, particularly effective cargo condensation, cellular uptake, DNA stability and prevention of degradation from nucleases as well as efficient delivery of DNA into the nucleus for expression [147-151]. The very first studies used DNAs natural structure as a blue print for designing AuNPs that are able to fulfill the necessary requirements, using spherical gold nanoparticles that are functionalized with amino acids thus resembling histones in size, shape and surface area. Several studies have shown lysine coated AuNPs produce more potent transfection vectors, that are able to condense DNA; for instance NP-LysG1 proved to be~28 more successful than polylysineheadgroups in reporter assays [111,149,150,152,153]. The superiority of lysine Dendron-functionalized AuNPs as delivery vectors can be attributed to their biometric design that makes their size similar to that of nucleosome core proteins (~6 nm) as well as forming electrostatic bonds with the phosphate backbone of DNA [149]. Modification of head groups attached to nanoparticles also serve to protect DNA from degradation cationic quaternary and trimythl ammonium-functionalized nanoparticles (NP-TMA) protected electrostatically bound plasmid DNA from DNase digestion. Most importantly, these additions are safe and display no cytotoxicity or unwanted immune responses [154,155]. The control of the transfection and release of nucleic acids, has been achieved by binding gold-thiolate on the surface of the AuNPs that are manipulated via intracellular glutathione levels. Ligands bound on the surface of the AuNPs are exchanged with the cellular glutathione (GSH), which will result in altering the AuNPs surface charge and loosening the nucleic acids bound to the nanoparticles [150]. Moreover, different GSH levels provide a mechanism for transfection regulation increasing efficiency in a concentration dependent fashion. Conversely, suppression of glutathione by L-buthionine-[S,R]-sulfoximine (BSO) treatment over 24 h caused lower transfection efficiency [154-157]. Furthermore, AuNPs may also be used in combination with other nano particles in order to improve and enhance efficiency. A more recent experiment combined both dendrimers and AuNPs, the unique morphology has maintained the three dimensional spherical form of dendrimers while increasing the number of binding sites [34,69]. These unique Au DENPs have a generation 5 PAMAM dendrimers with amine groups on their periphery, significantly augmented pDNA compaction and eventually improved nucleic acid delivery with a 100 times enhanced gene transfection efficiency than the conventional DENPs . Recently, a study using ethylenimine-conjugated AuNPs (PEI2-AuNPs) as a vector for corneal gene therapy demonstrated efficient delivery of BMP7 gene that significantly attenuated corneal fibrosis in an *in vivo* model. Furthermore, PEI2-GNPs exhibited minimal cytotoxicity and did not trigger an immune response [158] . More recently, a 2015 study have discussed the co delivery of and DNA and siRNA using hybrid coated gold nanoparticles [156,157].

Quantum dots for labeling genetic material: Quantum dots (QDs) are crystalline nanoparticles with electrical and mechanical properties. QDs are highly luminescent, colloidal semiconductor Nano crystals. QDs have unique size-dependant properties, which make them highly attractive for applications in catalysis, phosphors, photovoltaic, light emitting diodes (LEDs) and biological labeling. The main appealing feature of semiconductor NCs, are their mesoscopic properties that differentiates them from bulk crystals. Besides, it is possible to bind quantum dots to proteins and receptors to check with which molecules they interact and to explore their location in the cell. Hence, QDs are used in biomedical applications because of their unique tunable optical properties [12,16,155]. Made of semiconductor, quantum dots can be excited which makes them suitable not only in monitoring the genes; they are capable of overcoming the challenge of gene silencing. During excitation, the quantum dots attain a higher energy state. This usually occurs during preparation; before being used for gene delivery. However, upon entering the cell the differential pH causes the QDs return to a lower energy state. The photons lost during such process leads to fluorescence. The produced light bands are visible to the naked eye. They can be viewed eve within organic matter; that is, quantum dots have bioluminescence qualities. Therefore, their optical and electrical properties allow for bioluminescence. Their small sizes make them suitable for delivering genes; they can regain their sizes while in the cell. Semiconductor quantum dots (QDs) can be used to deliver genes such as RNA interference (RNAi) which is capable of silencing genes in the cell which either cause a disease or interfere with the activation of the delivered genes and synthesis of the therapeutic proteins [157-159]. The ability of the quantum dots to emit light in the visible spectrum of various wavelengths even within biological organisms make the nanoparticles important for tracking and monitoring the genes during the transfection. Such tracking and monitoring have provided important clues on how and when activation and silencing of the genes occur [160,161]. Moreover, they significantly reduce the degradation of the genes by the DNA nucleases. Additionally, QDs have been used as siRNA delivery vehicles to silence a target gene, and as fluorescent probes to analyze intracellular imaging *in vivo*. QDs-SiRNA complex has targeted HPV18 E6 oncogene which has eventually inhibited the growth of HeLa cells. QD-siRNA complexes serve as dual-modality; providing an optical and tool for live cell imaging and localization of QDs throughout the SiRNA delivery and transfection (Figure 9) [161].

Conclusion

In summary, gene therapy is one of the most exciting and revolutionary new approaches to therapies. The application of gene therapy has been hindered due to many reasons. It has been shown that therapeutic Nanomaterials could be utilized as promising tools to specifically deliver siRNA and mRNA to the target cells. Polymeric nanoparticles are the most commonly used type of nanoparticles used

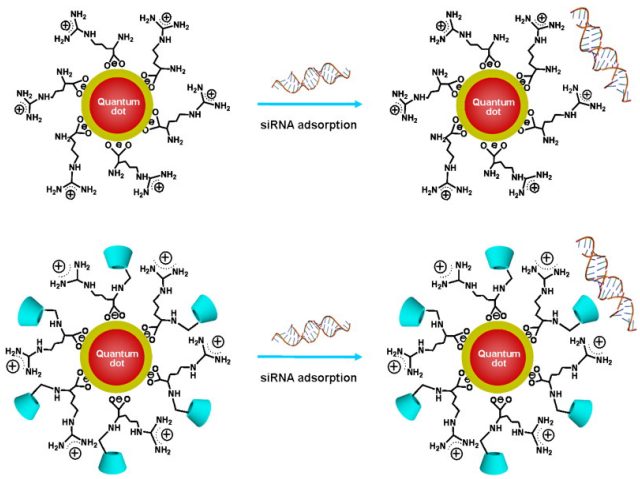

Figure 9: The adsorption of siRNA on the surface of differently functionalized QDs [161].

in gene therapy due to their biocompatibility and their ability to deliver the genetic material to its target with loss of its function. Nonetheless, the realization of such therapies is still debatable. Moving from the lab to the clinic has not yet been achieved. Hence, research in this area still requires in depth studies that involve functional assays. First, the nanomaterial should be designed and characterized; secondly, verify routes of administration of the therapies and finally, simplifying the synthesis methods making it trouble-free to expand at an industrial level.

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