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REVIEW ARTICLE

Nanovehicle-based Small Interfering RNA (siRNA) Delivery for Therapeutic Purposes: A New Molecular Approach in Pharmacogenomics

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Abstract: RNA interference (RNAi) is a process for regulating the gene expression in which small interfering RNAs (siRNAs) silence target genes. siRNA-based therapy as a new molecular treatment approach offers therapeutic prospects for many common diseases such as cancer and cardiovascular disorders. Nevertheless, the efficacy of siRNA delivery has, so far, remained a challenging issue. This is due to their easy degradation through the circulation system and the difficulties in the intracellular delivery to specific tissues where they silence the target genes. There have been many efforts to develop suitable, safe and effective siRNA delivery systems in the past decades. These efforts specifically aimed to protect siRNA from serum nucleases and deliver it to an intracellular region in the desired target cells. In this context, one of the new and popular approaches is nano vehicle-mediated siRNA delivery systems. The systems potentially may be used in future medicine, particularly for untreatable or poorly treated diseases. As we learn more about these delivery systems, we can better use the tremendous opportunities provided by siRNA-based therapeutics. The results of ongoing clinical trials will play an important role in determining whether siRNA-based drugs can be considered as a new class of drugs. Here, the authors reviewed and highlighted the recent advances in this exciting and fast-growing field to help in the development of effective therapeutic tools in controlling human diseases.

Keywords: Small interfering RNA; siRNA; drug delivery system; RNA interference; nanotechnology; pharmacogenomics

1. INTRODUCTION

RNA interference (RNAi) technology has attracted the attention of many researchers in recent years. RNAi is one of the best effective post-transcriptional processes for controlling gene expression levels in cells. When specific kinds of double-stranded RNAs (dsRNAs) are processed by enzymes called Dicer in the cytoplasm, small interfering RNAs (siRNAs) are formed and these novel RNAs are incorporated into the RNA-induced silencing complex (RISC) in order to regulate genes and genome [1]. The siRNA-RISC complex mediates process that eventually caused the degradation of related mRNA and silencing specific target genes, as illustrated in Fig. **1** [2]. Cancer, diabetes, cardiovascular disorders and many other common diseases are caused as a result of the abnormal gene overexpression or mutation. Hence reducing or silencing the expression of the related genes, particularly through new molecular and natural approaches, demonstrates a promising route for treatment of various human diseases, particularly those that are untreatable and those that have been less successful in treating them [3-5]. siRNA-based therapy is an effective approach that could potentially be useful for achieving these purposes. For instance, siRNA therapy in age-related macular degeneration and respiratory syncytial virus led to successful results with no serious adverse events [6, 7].

Despite the valuable achievements and a great therapeutic potential for many types of disorders, the major barrier against siRNA therapy is the efficiency of delivery to the desired position in the body. Naked siRNA could not only stimulate the innate immune responses but is also susceptible to enzymatic degradation [8, 9]. In addition, due to the size

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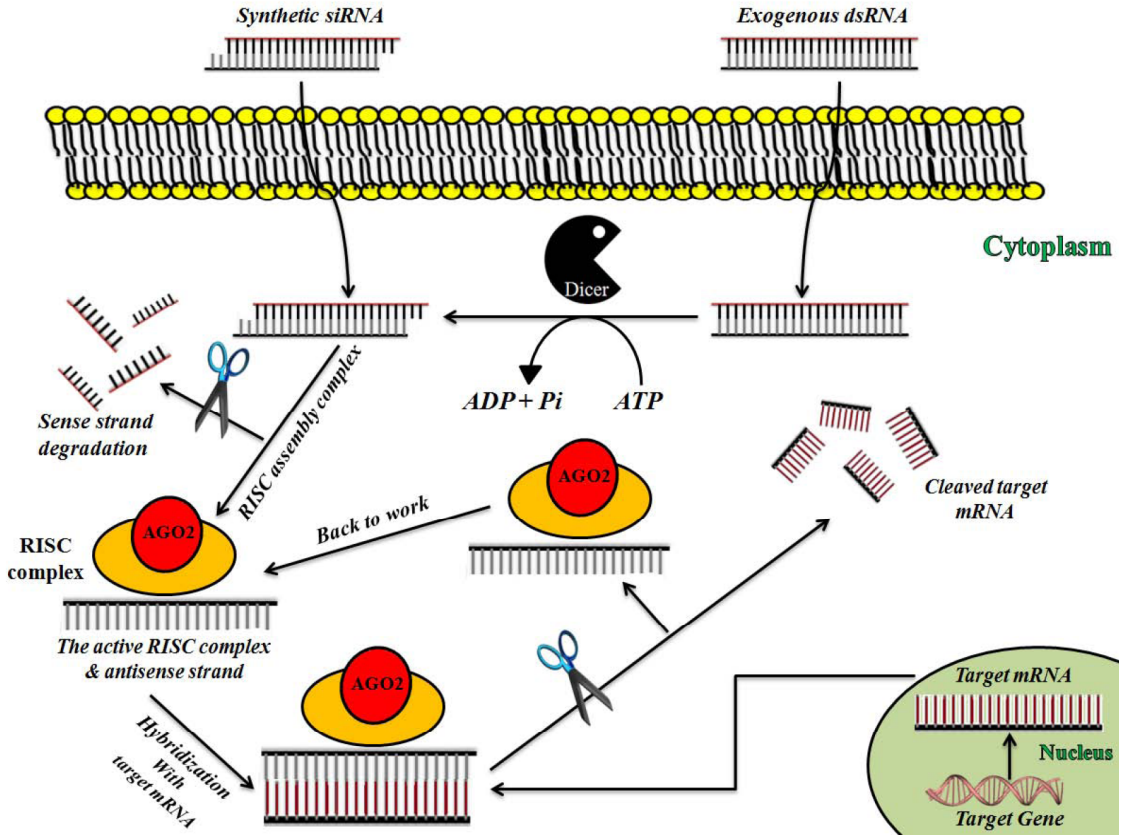

Fig. (1). Mechanism of mRNA degradation and gene silencing by siRNA. The cleavage of dsRNAs in the cytoplasm by the Dicer enzyme results in the production of siRNAs (short double-stranded oligonucleotides with 21-23 mer length and 2-nt 3′ overhangs). Synthetic siRNAs do not undergo Dicer editing and join the path automatically. The siRNA molecules are recognized by the complex RISC and subsequently, AGO2 cleaves the passenger or sense strand so that active RISC containing the guide or antisense strand is formed. The activated complex can search and hybridize to a target mRNA and inactivates the expression of its target gene by degrading the related mRNA. Abbreviations: dsRNA: double-stranded RNA, siRNA: small interfering RNA, RISC: RNA-induced silencing complex, AGO2: argonaute 2.

and negative charge of siRNA, there is a problem of passing through the cell membrane [10]. Also, when the unmodified siRNA is injected into the body, rapid accumulation of siRNA in the kidney and intestine will lead to a decrease in its bioavailability [11].

The effective drug treatment processes have principles such as having appropriate concentrations in the delivery target cell and transferring the proper form of drug to a specific site. Therefore, it is very important to protect the integrity of siRNA during transmission to the exact location and eventually delivering into the target cell with high efficiency. siRNA delivery systems can be categorized by physical methods, conjugation methods, natural carrier (viruses and bacteria) and nonviral carrier methods. Among these strategies, the use of nanoparticles due to the highly tunable features can be invaluable. Many researchers investigated the potential ability of nanoparticles for siRNA-based drug therapy and stated that this approach could be a safe and efficient way with several advantages [12-18]. Some RNAi-based drugs that have been used in clinical trials are listed in Table **1**.

2. NANOCARRIERS FOR siRNA DRUG DELIVERY

Thus far, different nanostructures to deliver siRNA to the target cells have been used, including noncomplex polymers, lipid-based nanomaterials, gold nanoparticles, magnetic nanoparticles, mesoporous silica nanoparticles, and carbon nanomaterials. In the following, we briefly review some of these nanocarriers.

2.1. Polymer-based Carriers

Many attempts have been made to improve the chemical design of some of the classical transfection agents such as polylysine and polybrene. These agents are polycations that are able to establish electrostatic interactions with the negatively charged cell membrane [19]. Generally, the negative charge of siRNA causes polymers with cationic portions to easily form a complex *via* electrostatic interactions. Meanwhile, the higher amount of nanoparticles may be required for electro-neutralization of the negative charge of siRNA [20]. Factors such as size, surface charge, and structure of polymer/siRNA complexes can play a role in modulating the properties of these complexes through regulating the ratio of positive charge in the polymer to negative charge in siRNA [21]. Among various cationic polymers, synthetic polymers, including polyethyleneimine, poly-L-lysine, and cyclodextrin-based polycations, along with natural polymers such as chitosan and atelocollagen have already been used as siRNA delivery carriers. Some of the applications of these polymers in siRNA delivery are discussed below.

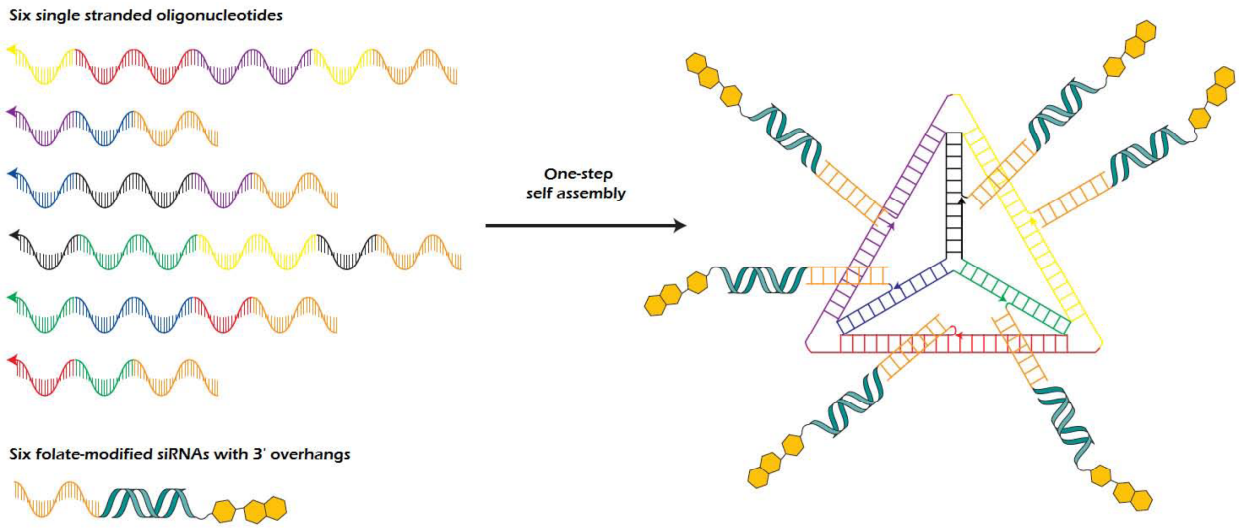

Fig. (2). The advanced delivery systems formed by self-assembly of oligonucleotide nanoparticles. DNA tetrahedra carrying six siRNAs were produced by hybridization of complementary strands [adopted with permission from Kanasty *et al*. (Delivery materials for siRNA therapeutics. Nature materials 2013; 12: 967-77)].

2.1.1. Polyethyleneimine (PEI)

PEI demonstrates the high transfection efficiency of siRNA delivery. However, a major problem with PEI is cytotoxicity, which can correlate with molecular weight and the number of branches. It has been demonstrated that low molecular weight PEI indicates increased transfection efficiency and as well as reduced cytotoxicity [22-24]. Branched PEI (bPEI) suffers from significant toxicity caused by inflammatory responses and is often subjected to rapid macrophage uptake and clearance. In this context, PEGylation can be considered because it contributes to decreased uptake by the reticuloendothelial system or macrophages, which lead to an increase in half-life in the blood. PEGylated PEI/siRNA nanoparticles illustrated an obviously reduced tendency to aggregate [25].

Although PEG-PEI particles indicated decreased cytotoxicity, hydrophobic fatty acid-modified PEG-PEI nanoparticles and PEI particles invoked inflammatory cytokines and caused the increased concentrations of IgM in the bronchoalveolar fluid. The modified PEI particles exhibited the greatest knockdown efficiency in leukocytes and alveolar macrophages, so it seems that further investigations are required to achieve the improved knockdown efficiency, low cytotoxicity, and reduced immunostimulatory activities using the beneficial modifications in the PEI-based carriers [11]. Accordingly, Nimesh *et al* showed that acylated PEI nanoparticles with propionic anhydride followed by cross-linking with derivatized PEG could be efficiently used as siRNA delivery system with reduced cytotoxicity [20]. In a recent study, siRNA with PEI as a delivery agent is used against Beclin1 gene for controlling HIV replication. siRNA complexed with PEI was administered intranasally in adult mouse brain. This approach could potentially offer an efficient means of gene silencing-mediated therapy in the HIV-infected brain [26]. Moreover, other studies presented examples of the successful use of PEI for transferring siRNA as a drug in the treatment of cardiovascular disease/injury and in the direct oncogene targeting in many cancers [18, 27].

2.1.2. Poly (DL-lactide-co-glycolide)

Poly (DL-lactide-co-glycolide) (PLGA) as an FDA-approved biodegradable polymer has recently been used for siRNA and functional plasmid DNA (pDNA) delivery [2]. Due to the solid form of PLGA nanoparticles (unlike lipid and polyplexes), they are more stable and also capable of protecting siRNAs from degradation during circulation in the bloodstream [28]. An obvious trouble for PLGA is the anionic structure of these nanoparticles. To achieve the positive charge, the surface of PLGA nanoparticles can be decorated with acetyl derivative PEI, which improves surface fictionalization and siRNA delivery capacity [29]. Adding a small amount of PEI to the PLGA polymer phase ameliorates the encapsulation and release of siRNA [30]. Compared with unmodified PLGA nanoparticles, chitosan-modified PLGA nanoparticles showed much higher encapsulation efficiency and also were more effectively taken up by the cells. Also, the gene silencing efficiency of chitosan-modified PLGA nanoparticles was higher and more prolonged than unmodified PLGA and naked siRNA [31]. Tiwari *et al*. [32] demonstrated that curcumin-encapsulated PLGA nanoparticles can induce the proliferation and differentiation of neural stem cells that may be a useful therapeutic approach to treating neurodegenerative diseases such as Alzheimer's disease. The hybrid nanoparticles of PLGA and lipids have also been investigated in some studies. For instance, Wang *et al*. [33] described a versatile platform for siRNA delivery based on PLGA-PEG–cationic lipid nanoparticles, which were synthesized using the double emulsion method. The resulting nanoparticles had a high encapsulation efficiency of siRNA (up to 90%) and demonstrated the effective downregulation of the target genes *in vitro* and *in vivo*.

2.1.3. Chitosan

Chitosan is a cationic polymer and a versatile candidate in gene delivery that is derived from the most abundant nitrogen-bearing organic compound found in nature, the linear polymer chitin, by alkaline or enzymatic deacetylation. It is

Table 1. RNAi-based drugs that have been investigated in clinical trials.

Drug	Delivery System	Disease	Phase	Status	Company	Clinical trials.gov identifier
ALN–VSP02 *	LNP	Solid tumours	I	Completed	Alnylam Pharma-ceuticals	NCT01158079
siRNA–EphA2–DOPC *	LNP	Advanced cancers	I	Recruiting	MD Anderson Cancer Center	NCT01591356
Atu027 *	LNP	Solid tumours	I	Completed	Silence Therapeutics	NCT00938574
TKM–080301 *	LNP	Cancer	I	Completed	Tekmira Pharmaceutical	NCT01262235
TKM–100201 *	LNP	Ebola-virus infection	I	Terminated	Tekmira Pharmaceutical	NCT01518881
ALN–RSV01 *	Naked siRNA	Respiratory syncytial virus infections	II	Completed	Alnylam Pharmaceuticals	NCT00658086
PRO-040201 *	LNP	Hypercholesterolaemia	I	Terminated	Tekmira Pharmaceutical	NCT00927459
ALN–PCS02 *	LNP	Hypercholesterolaemia	I	Completed	Alnylam Pharmaceuticals	NCT01437059
ALN–TTR02 *	LNP	Transthyretin-mediated amyloidosis	II	Completed	Alnylam Pharmaceuticals	NCT01617967
CALAA-01 *	Cyclodextrin NP	Solid tumours	I	Terminated	Calando Pharmaceuticals	NCT00689065
TD101 *	Naked siRNA	Pachyonychia congenita	I	Completed	Pachyonychia Congenita Project	NCT00716014
AGN211745 *	Naked siRNA	Age-related macular degeneration, choroidal neovascularization	II	Terminated	Allergan	NCT00395057
QPI-1007 *	Naked siRNA	Optic atrophy, non-arteritic anterior ischaemic optic neuropathy	I	Completed	Quark Pharmaceuticals	NCT01064505
I5NP *	Naked siRNA	Kidney injury, acute renal failure Delayed gaft function, complications of kidney transplant	I I, II	Completed Completed	Quark Pharmaceuticals Quark Pharmaceuticals	NCT00554359 NCT00802347
PF-655 (PF-04523655) *	Naked siRNA	Choroidal neovascularization, diabetic retinopathy, diabetic macular oedema	II	Completed	Quark Pharmaceuticals	NCT01445899
siG12D LODER *	LODER polymer	Pancreatic cancer	II	Recruiting	Silenseed	NCT01676259
Bevasiranib *	Naked siRNA	Diabetic macular oedema, macular degeneration	II	Completed	Opko Health	NCT00306904
SYL1001 *	Naked siRNA	Ocular pain, dry-eye syndrome	I, II	Completed	Sylentis	NCT01776658
SYL040012 *	Naked siRNA	Ocular hypertension, open-angle glau-coma	II	Completed	Sylentis	NCT01739244
CEQ508 *	Escherichia coli-carrying shRNA	Familial adenomatous polyposis	I, II	Recruiting	Marina Biotech	Unknown
RXi-109 *	Self-delivering RNAi compound	Cicatrix scar prevention	I	Completed	RXi Pharmaceuti-cals	NCT01780077

(Table 1) contd….

Drug	Delivery System	Disease	Phase	Status	Company	Clinical trials.gov identifier
ALN–TTRsc *	siRNA–GalNAc conjugate	Transthyretin-mediated amyloidosis	I	Completed	Alnylam Pharmaceuticals	NCT01814839
ARC-520 *	DPC	HBV	I	Completed	Arrowhead Research	NCT01872065
DCR-MYC	LNP	Solid Tumors, Multiple Myeloma, Non-Hodgkins Lymphoma	I	Terminated	Dicerna Pharmaceuticals	NCT02110563
ND-L02-s0201 Injection	siRNA lipid nanoparticle conjugated to Vitamin A	Moderate to Extensive Hepatic Fibrosis	I	Completed	Nitto Denko	NCT02227459
DCR-MYC	LNP	Hepatocellular Carcinoma	I, II	Terminated	Dicerna Pharmaceuticals	NCT02314052
TKM-080301	LNP	Primary and secondary liver cancers	I	Completed	National Cancer Institute	NCT01437007
ALN-VSP02	LNP	Solid Tumors	I	Completed	Alnylam Pharmaceuticals	NCT00882180
APN401	Ex vivo transfection	Solid Tumors	I	Completed	Wake Forest University Health Sciences	NCT02166255

DPC, dynamic polyconjugate; LNP, lipid nanoparticle; NP, nanoparticle; shRNA, short hairpin RNA. *Adopted with permission from Kanasty *et al*. (Delivery materials for siRNA therapeutics. Nature materials 2013; 12: 967-77).

biocompatible and biodegradable with the low toxicity rate and its positive charge allow its binding to DNA. This binding provides protection from harmful nucleases which potentially transforms chitosan into a very promising carrier for delivery of siRNA to the living cells. Moreover, this complexation results in nanoparticles, which are more susceptible to enter the cell due to their small size. The biodegradability of polymer also ensures a controlled siRNA release [29, 34].

Chitosan has mucoadhesive properties and exhibits the property of mucosal gene silencing in the complex with siRNA. This property is a result of electrostatic interaction between an amine on the polymer chain and sialic acid residue on the glycoprotein mucin [35, 36]. Some attempts were made for siRNA delivery to the lungs, either as naked or by nanoparticles. Howard *et al*. introduced a pulmonary delivery system of siRNA by chitosan nanoparticles *via* the nasal route [36]. According to studies, naked siRNA exhibits rapid renal clearance, with a circulatory half-life of less than 5 minutes that could be increased to more than 30 minutes by conjugation with chitosan and cholesterol [37].

Despite the advantages of chitosan including mucoadhesivity, biocompatibility, biodegradability, and low cost of production, there is a big problem with the use of high molecular weight chitosan and its toxicity. The toxicity has limited the application of chitosan in clinical trials. However, chitosan has yet to be been used for carrying siRNA molecules in many investigations. These included a broad range from delivery of IGF-1R siRNA in lung cancer cells to BACE1 siRNA for Alzheimer's patients or stimulating the anti-inflammatory effects on macrophages for potential treatment of inflammatory disorders [38-40].

2.1.4. Cyclodextrin Polymers (CDP)

Cyclodextrins (CDs) are cyclic oligosaccharides that consist of five or more α-D-glucopyranoside units. Since CD does not stimulate the immune system, and its toxicity is low, it has been used in pharmaceutical applications [41]. Davis *et al*. reported the first mechanistic evidence of siRNA effect observed in Phase I clinical trial when a siRNA was employed against the M2 subunit of ribonucleotide reductase (RRM2) as an established anti-cancer target using a nanoparticle delivery system. A cyclodextrin-based polymer was one of the components of this delivery system [14]. In another study, Godinho *et al*. showed the application of CDP-based nanoparticles in the siRNA drug delivery. The CDs-based nanosize particles synthesized by these researchers were stable in an artificial cerebrospinal fluid. Their findings indicated that the CD-siRNA complexes are capable of reducing the expression of the Huntingtin gene in rat striatal cells and human Huntington's disease primary fibroblasts [42]. Additionally, it has been shown that polycationic cyclodextrin nanoparticles containing siRNA could be used for mesangial delivery of siRNA in humans [43].

2.2. Gold Nanoparticles (AuNPs)

The physical properties of AuNPs have caused them to have many applications in medicine [44]. AuNPs have several advantages for use as a core, including bio-inertia, ready synthesis, nontoxicity, and easy functionalization, which make them attractive scaffolds for the generation of transfection agents [45]. Gold can be incorporated into polymeric nanoparticles or liposomes which deliver large payloads [46]. Covalent attachment of nucleic acids to AuNPs is an effective approach for specific gene silencing and/or transporting oligonucleotides. The modification does not inhibit the biological activity of nucleic acids. AuNPs covalently attached with thiol-modified oligonucleotides can be used for siRNA-based gene silencing. In this approach, the dense shell of oligonucleotides on the NPs surface inhibits their degradation by nucleases and lead to protection of the drug shipment [47]. In a study, Han *et al*. [45] first generated a positively charged AuNP-Cs core, then deposited the pH-

responsive charge-reversible polymer (PAH-Cit) and PEI onto the surface of AuNP-CS to form a PEI/PAH-Cit/AuNP-CS shell/core structure. This structure indicated a high siRNA binding affinity and was able to release as much as 79% of the loaded siRNA at pH 5.5 *in vitro*.

It has also been indicated that coating gold nanoparticles with amino acid-based head groups could generate effective vectors. These amino acid-based nanoparticles can be responsive to intracellular glutathione concentrations, a feature that plays a role in the controlled release of nucleic acids [48]. The light-regulated release is another controlled releasing approach. In this method, DNA release occurs in a photochemical way from complex using near-ultraviolet irradiation (>350 nm) [49].

In addition to covalently functionalized AuNPs, noncovalent nucleic acid delivery vehicles can be an attractive alternative to covalent systems. These systems make it possible to have multiple options for vehicle design, such as amino acid–functionalized AuNPs (AA–AuNPs), mixed-monolayer-protected AuNPs (MM-AuNPs), and layer-by-layer-fabricated AuNPs (LbL-AuNPs) [47]. The conjugated AuNPs-siRNA has been used to achieve a variety of purposes in the treatment of human diseases, including elevating the serum stability of siRNA [50], stimulating and initiating of the mitochondrial apoptosis pathway for cancer cell demolition [51], targeting knockdown of a specific gene target in Hela cells [52], and knockdown of some specific mutations to decrease downstream signals and cell viability in uveal melanoma cells [53]. Nonetheless, it should be mentioned that some issues have to be solved before these conjugates are used to treat human diseases, including minimizing cytotoxicity, minimizing side effects through targeting to specific organs and tissues and exploring immunological issues [47].

2.3. Lipid-based Nanoparticles (LNPs)

Lipid nanoparticle (LNP) is now a common general term used to describe lipid-based delivery systems with diameters in the range of 25 to 150 nm. Liposomes have been considered by researchers for decades as the carriages of DNA-based drugs and other components in many fields, particularly cancer therapy. This is attributed to the excellent capabilities of liposomes as nucleic acid delivery systems, including protection from renal clearance, shielding oligonucleotides from nuclease degradation, promoting cellular uptake and endosomal escaping [54-59]. These functions were related before their use as a siRNA delivery vehicle. The first activity of liposomal siRNA formulations was reported in non-human primates [60]. Since then, a number of LNP siRNA drugs have entered clinical trials, including targeting treatments for hypercholesterolaemia, transthyretin-mediated amyloidosis and cancer [61-63].

Cationic or ionizable lipids are mainly liposomes used for siRNA transportation. Positively charged lipids have some benefits that include improving the capture of negatively charged siRNA, increasing cellular uptake, and helping in endosomal escaping. In general, the composition of these lipids is divided into three parts: an amine head group, a linker group and hydrophobic tails [61, 64]. Cationic liposomes have been used as antigen delivery vesicles in vaccines [65]. Increased efficacy of doxorubicin (DOX) drug delivery against multidrug-resistant (MDR) lung cancer cells has been reported by Saad *et al*. The researchers developed a cationic liposome, which could release DOX and siRNAs simultaneously. The co-delivery of DOX and siRNA was an effective tool in cell-death induction and suppression of cellular resistance in MDR lung cancer cells [66].

Many of the lipid-based delivery vehicles self-assemble with siRNA through electrostatic interactions with positively charged amines. Several features can influence the effective siRNA delivery, including the use of cationic or ionizable lipids, shielding lipids, cholesterol and targeting ligands [67]. Liposome internalization into cells occurs through endocytosis and the release of siRNA or pDNA take place through endosomal escape. The size of the liposome is important and should be < 100 nm in diameter to be considered as a carrier. The larger particles tend to be taken up by Kupffer cells or other components of the reticuloendothelial system (RES) [67, 68].

PEGylation of liposomes can reduce their RES uptake and increase their circulation time. In the absence of PEG-lipids, particle-particle crosslinking and aggregation can grow nucleic acid-containing LNPs to unsuitable sizes for intravenous delivery. PEG-coated Bcl2 siRNA-lipoplex was exploited to downregulate *Bcl2* expression in the 5-Fluorouracil resistant DLD-1 cell line [69]. It should be mentioned that the fusion of LNPs with target membranes is dependent on the level of negative charge on the target [70]. LNPs showed some valuable applications in the field of medicine, including the modification of lipid profile and reduction of low density lipoprotein and triglyceride in preclinical disease models, which would be a promising approach for diabetic dyslipidemic patients [71, 72], silencing target genes in hepatocytes to treat transthyretin-induced amyloidosis patients [62], hypercholesterolemia treatment [61], direct inhibition of β-catenin in tumors with different origin such as liver, hepatocellular and colorectal cancers [63], and therapeutic advancements in iron overload diseases associated with reduced hepcidin expression [73].

2.4. Dendrimer-based Carriers

Dendrimers are highly branched and star-shaped macromolecules in nanoscales. They are characterized by three main components, including a central core, an interior dendritic structure (the branches), and an exterior surface with functional surface groups. The varied combination of these components yields products with different shapes and sizes that are ideal candidates for applications in both biological and materials sciences. The interaction of positively charged dendrimer branches with siRNA has been investigated in the field of siRNA and antisense oligonucleotide delivery. The ability of dendrimers to interact with siRNA is dependent on the generation of polymers and pH [74, 75].

Among the various dendrimers, poly(amidoamine) (PAMAM) dendrimers have become more prominent. This was because of their intrinsic features such as molecular flexibility and atomic structure. Different generations of PAMAM dendrimers exhibit various behaviors in binding siRNA. While G4 (fourth generation) demonstrates high-quality interaction to siRNA, a G6 generation has less binding affinity. G5 indicates a hybrid behavior, maintaining

rigid and flexible features, with a strong dependence on the environmental pH [74]. G4 generation of PAMAM-paclitaxel conjugates have the internal cationic charges that are suitable for interaction with siRNAs. After exposure to the body, these nanoparticles accumulated in the tumor tissues and internalized into the cells effectively. This causes a decrease in the side effects of chemotherapy drugs [74, 75]. Different types of dendrimers have been employed for the target tissue or disease. Based on former investigations, the capabilities of dendrimers emphasize their potential for further applications in functional genomics and therapeutic strategies [76, 77]. The nanocarriers have been used for siRNA gene therapy in cardiovascular defects, lung vasculature disorders, a deleterious virus targeting in neurons, and types of cancers [78-82].

2.5. Magnetic Nanoparticles-based Carriers

Nucleic acid delivery under the magnetic field influences the nucleic acid carriers, which are associated with magnetic particles. Magnetofection is an approach to increase transfection efficiency that is widely used to transfer oligonucleo-tides, such as the miRNAs and siRNAs (Table 2). Recently, Singh *et al*. [83] reported that magnetofection can be a promising novel approach of *in vivo* gene delivery for targeted therapy in rectoanal motility disorders that as well as potentially may spread to treatment in other organs. Magnetofection is a reliable method with low cytotoxicity for siRNA delivery into hard to transfect cells and it is an advantage for functional endpoint analyses of gene silencing such as analysis of enzyme function [84].

2.6. Porous Silicon (PSi)-based Carriers

The biomedical applications of porous silicon materials have widely spread since the first studies were reported. These nanostructures are multifunctional and versatile platforms that are used in nanomedicine to effectively deliver drugs and nucleic acids for therapy purposes [85, 86]. Meng *et al*. prepared mesoporous silicon nanoparticles (MSNP) functionalized by phosphonate groups, which allowed electrostatic binding of DOX to the porous spaces [85]. Also, MSNP modified with PAMAM dendrimers (G2) was investigated by Chen *et al*. This approach led to an increase in the anticancer efficacy of DOX by 132 fold compared to free DOX in multidrug resistant ovarian cancer cells [86]. Targeting the brain tissue and delivery of siRNAs to defective neuron cells [87], combination therapy in melanoma lung metastasis and enhancement of other therapeutic agents in target cells [88], siRNA delivery to reduce the expression of *MRP1* gene, which results in a significant reduction in the glioblastoma cell proliferation [89], and selective silencing of tumor necrosis factor receptor-1 in human lung microvascular endothelial [90] are some of the recent valuable utilities of PSi as a siRNA delivery vehicle to the cells.

2.7. Super Carbonate Apatite (sCA)-based Carriers

Super carbonate apatite is the smallest class of nanocarriers consisting of inorganic ions that accumulate particularly in tumors. These nanoparticles are highly stable at pH 7.4 but easily degradable at acidic pH that exists in the endosomal compartments of cancer cells. CA shows nearly 10–100-fold more transfection efficiency of DNA *in vitro* compared to Lipofectamine 2000 (Lp) or calcium phosphate precipitation in mammalian cells [91]. Li *et al*. [92] developed sCA nanoparticles, which were able to co-deliver two kinds of siRNA targeting two genes of ATP-binding cassette (ABC) transporter (ABCG2 and ABCB1) in human breast cancer cells to overcome multidrug resistance of cancer. In addition, according to studies, sCA-survivin-siRNA can induce apoptosis in tumor cells and also inhibit tumor growth, thus it could be useful as an innovative delivery system for siRNA in the treatment of solid tumors [91].

Table 2. Investigations that have used the magnetofection technique for siRNA delivery.

Research	Refs.
siRNA delivery to primary endothelial cells	[93]
siRNA delivery to myofibroblasts	[94]
siRNA transfection in primary culture of rat embryonic DRG (E14)	[95]
β-Catenin siRNA transfection in a rat fibroblast cell line (3Y1)	[96]
Induced gene knockdown by siRNA in the endothelial cell line HMEC-1 (allowed researchers to figure out the implication of a Rho kinase (ROCK-II isoform) in the formation of microparticles in response to thrombin stimulation)	[97]
siRNA transfection in suspension cells such as MOLT-4 and Jurkat Human T cell leukemia [which permitted to show the implication of RCAS1 (a receptor-binding cancer antigen) in T cell apoptosis induced by HIV infection]	[98]
Cell signaling pathway modifications	[99, 100]
Ultrasound-enhanced siRNA delivery for apoptosis induction	[101]
siRNA delivery into the cytoplasm of breast cancer cells (MCF-7) and apoptosis induction	[102]
Direct delivery of functional siRNA into the cytoplasm of human osteosarcoma cancer cells	[103]
Triggering cell death by silencing of some deleterious genes in specific cancers	[104]

CONCLUSION

Nanoparticles are extensively studied structures for siRNA delivery systems which potentially may be used in future medicine, particularly for untreatable or poorly treated diseases. As we learn more about these systems *in vitro* and *in vivo*, we can better use the tremendous opportunities provided by siRNA-based therapeutics. In this context, some clinical trials have achieved promising results. However, there are challenges ahead of this fast-growing field that we must overcome, including toxicity, instability in circulation, unsuccessful targeting, undesirable immune responses, and difficulties in the delivery efficiency. The results of ongoing clinical trials will play an important role in determining whether siRNA-based drugs can be considered as a new class of drugs.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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