Expert Opinion

Recent advances of siRNA delivery by nanoparticles

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Introduction: The field of RNA interference technology has been researched extensively in recent years. However, the development of clinically suitable, safe and effective drug delivery vehicles is still required.

Areas covered: This paper reviews the recent advances of non-viral delivery of small interfering RNA (siRNA) by nanoparticles, including biodegradable nanoparticles, liposomes, polyplex, lipoplex and dendrimers. The characteristics, composition, preparation, applications and advantages of different nanoparticle delivery strategies are also discussed in detail, along with the recent progress of non-viral nanoparticle carrier systems for siRNA delivery in preclinical and clinical studies.

Expert opinion: Non-viral carrier systems, especially nanoparticles, have been investigated extensively for siRNA delivery, and may be utilized in clinical applications in the future. So far, a few preliminary clinical trials of nanoparticles have produced promising results. However, further research is still required to pave the way to successful clinical applications. The most important issues that need to be focused on include encapsulation efficiency, formulation stability of siRNA, degradation in circulation, endosomal escape and delivery efficiency, targeting, toxicity and off-target effects. Pharmacology and pharmacokinetic studies also present another great challenge for nanoparticle delivery systems, owing to the unique nature of siRNA oligonucleotides compared with small molecules.

Keywords: drug delivery, gene delivery, nanoparticle, non-viral vector, RNA interference, siRNA

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1. Introduction

Long, double-stranded RNAs (dsRNAs) were first shown to mediate RNA interference (RNAi) in *Caenorhabditis elegans*, and the potential use of RNAi for human therapy has been demonstrated by the finding that small interfering RNAs (siRNAs; ~ 21-base pair double-stranded RNA) can elicit RNAi in mammalian cells without producing an interferon response. The field of Nobel Prize-winning RNAi technology is one that has been researched extensively in biotechnology in recent years [1]. Small interfering RNAs, which are cleavaged from dsRNA by dicers, are incorporated into the RNA-induced silencing complex (RISC), which mediates mRNA sequence-specific binding and cleavage. As a result, mRNA transcription is halted and consequently the expression of specific target genes is silenced (**Figure 1**). Small interfering RNA has not only been widely used as a valuable tool for functional genomics research, but it also has demonstrated great potential in biomedical therapeutic applications for diseases caused by abnormal gene overexpression or mutation, such as cancer treatment, diabetes, pain management and gene probing. Today, there are promising data from continuing clinical trials for the treatment of age-related macular degeneration and respiratory syncytial virus by using siRNA. Despite these early successes, the widespread use of RNAi therapeutics

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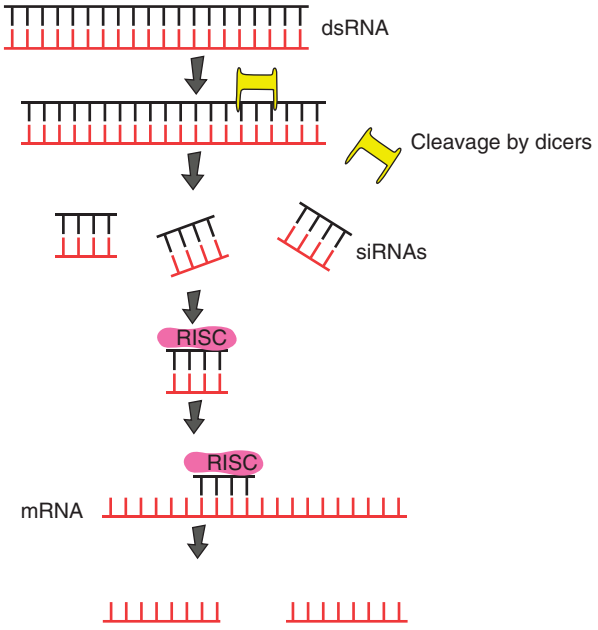

Figure 1. The mechanism of RNA interference showing cleavage of dsRNA into siRNA by dicers and formation of RISC.

dsRNA: Double-stranded RNA; RISC: RNA-induced silencing complex; siRNA: Small interfering RNA.

for disease prevention and treatment requires the development of clinically suitable, safe and effective drug delivery vehicles [2].

At present, delivery strategies can be categorized into physical methods, conjugation methods, and viral and non-viral carrier-mediated methods. Physical techniques can enhance siRNA uptake at a specific tissue site using electroporation, pressure, mechanical massage, and so on. Terminal modification of siRNAs can enhance their resistance to degradation by exonucleases in serum and tissue. Moreover, modification with a suitable ligand can achieve targeted delivery [3]. Non-viral carrier systems, which include nanoparticles, liposomes, lipoplex, polyplex and receptor-targeted nanocomplex (RTN), are the most promising delivery methods. They have shown improved *in vivo* stability, target specificity, and cell/tissue uptake and internalization of the encapsulated RNAi oligonucleotides, which result in more effective silencing with less cellular toxicity and immune stimulation. 'Stealth' systems are important for the clinical applications, as most successful nano-size delivery systems are for passive targeting rather than for active targeting of tissue. [4]. Therefore, non-viral gene delivery has been gaining considerable attention recently. Although the transfection efficacy is low in non-viral vector-mediated gene transfer compared with viral ones, non-viral vectors are relatively easy to prepare, less immunogenic and oncogenic, and have no potential of virus recombination and no limitation on the size of a transferred gene [5].

In addition, developments in nanotechnology now offer numerous non-viral vectors that have been fabricated and found to be capable of transmitting the biopharmaceuticals into the target cell and even into specific subcellular compartments such as mitochondria. Despite the progress of physical methods (i.e., ultrasound, electroporation), chemical methods have been shown to accomplish high-level and safe transgene expression [6]. Besides siRNA, short hairpin RNA (shRNA)-expressing vectors have been explored as alternative strategies for both local and systemic delivery [7]. Antibody, peptide and aptamers have been used to assist targeted siRNA nanoparticle delivery by specifically recognizing and tightly binding their cognate targets by means of well-defined secondary and three-dimensional structures [8]. Nanoparticle-siRNA conjugates have emerged as potential delivery vehicles. The accessibility of the siRNA linked to the nanoparticle and the lability of the crosslinker have been shown to be critical for efficient gene knockdown [9]. Electrostatic surface modifications were also used to prepare multilayer nanoparticles to improve siRNA delivery. These modifications have been used to stabilize therapeutics *in vivo*, add cell-specific targeting ligands and promote controlled release [10].

At present, there are very few *in vivo* studies using siRNA by itself, because this particular substance needs to overcome the body's defense mechanisms in order to be introduced into the specific gene sequence. As there are so many barriers that the human body presents it is imperative to look into different delivery systems for siRNA. Despite these hurdles, several polymer nanocarriers have been tested to deliver active RNA interfering effectors *in vivo*, making possible their administration by the intravenous route [11,12]. For example, using liposomes, nanoparticles and carbon nanotubes to deliver cancer drugs and siRNA, and antibody has been a success in recent preclinical trials of targeted drug delivery in pancreatic cancer [13]. Pharmacodynamics and pharmacokinetics of siRNA delivery within the setting of the wide variety of *in vivo* animal models have also been studied [14].

In this review, the recent advances, mainly in the past 2 years, of non-viral delivery of siRNA by nanoparticles, including biodegradable nanoparticles, liposomes, polyplex, lipoplex, dendrimers, and so on, are focused on. The characteristics, composition, preparation, applications and advantages of different nanoparticle delivery strategies are discussed in detail.

2. Nanoparticle carrier systems for siRNA delivery

2.1 Poly(dl-lactide-*co*-glycolide) nanoparticle

Poly(DL-lactide-*co*-glycolide) (PLGA) is an FDA-approved biodegradable polymer. PLGA nanoparticle has been used as a gene vector for siRNA and functional plasmid DNA (pDNA) delivery in recent years. Biodegradable PLGA nanoparticles were prepared to load siRNA oligonucleotides

with the desired physicochemical properties, as demonstrated in **Figure 2**. The green fluorescent protein (GFP) siRNA oligonucleotides were successfully loaded into PLGA nanoparticles and delivered in 293T cells. Compared with conventional carrier systems, the biodegradable polymeric nanoparticle system offers improved formulation stability, which is practically beneficial and may be used in future clinical studies of siRNA therapeutics [15]. The siRNA can be encapsulated in the core of PLGA nanoparticles by the double-emulsion solvent evaporation method. An encapsulation efficiency of up to 57% was achieved by adjusting the inner water phase volume, the PLGA concentration, the first emulsification sonication time and stabilization of the water–oil interface with serum albumin [16]. Potential problems for PLGA nanoparticles are the efficient endosomal escape and timely release of encapsulated siRNA. Therefore, PLGA nanoparticles are sometimes modified to have more efficient endosomal escape and better release profile, which are discussed later.

Small interfering RNA-loaded PLGA nanoparticles have been studied recently for various types of cancer. PLGA nanoparticles were used to deliver simultaneously anticancer drug, paclitaxel, along with P-gp-targeted siRNA to overcome tumor drug resistance. Nanoparticles were surface-functionalized with biotin for active tumor targeting. Dual-agent nanoparticles encapsulating the combination of paclitaxel and P-gp-targeted siRNA showed significantly higher cytotoxicity *in vitro* than nanoparticles loaded with paclitaxel alone. *In vivo* studies in a mouse model of drug-resistant tumor demonstrated greater inhibition of tumor growth following the treatment with biotin-functionalized nanoparticles encapsulating both paclitaxel and P-gp-targeted siRNA at a paclitaxel dose that was ineffective in the absence of gene silencing [17]. PLGA nanoparticles loaded with pDrive-sh AnxA2 plasmid DNA is capable of sustained intracellular delivery of pDrive-sh AnxA2 plasmid DNA vector for long-term siRNA-mediated downregulation of annexin A2. Intratumoral administration of pDrive-sh AnxA2-loaded nanoparticles to xenograft prostate tumors in nude mice demonstrates an overall decrease in tumor growth [18]. Methyl-CpG binding domain protein 1 (MBD1) is a transcriptional regulator that binds methylated CpG islands of tumor suppressor genes and represses their transcription. MBD1 siRNA plasmid loaded into poloxamer-modified PLGA nanoparticles can be successfully transfected into pancreatic tumor cells, and the MBD1 nanoparticle compound can inhibit cell growth and induce apoptosis [19].

Local delivery of siRNA to the lungs constitutes a promising new area in drug delivery. PLGA nanoparticles were investigated for inhalation therapy prepared by a spray-drying method and optimized by statistical methods. The use of mannitol in the formulation allowed a significantly lower moisture content than trehalose and lactose. The integrity and biological activity of the siRNA were preserved during the spray-drying process [20].

One problem for PLGA nanoparticles is that nanoparticles are anionic. To achieve positive charge, the surface of PLGA nanoparticles can be decorated with polyethyleneimine (PEI) using a cetyl derivative to improve surface fictionalization and siRNA delivery. Sub-micrometer particles were produced by an emulsion-diffusion method using benzyl alcohol. The modified particles were able to bind and mediate siRNA delivery into the human osteosarcoma cell line U2OS and the murine macrophage cell line J774.1 [21]. To improve siRNA delivery for possible clinical applications, PLGA nanoparticles were also modified with biocompatible and biodegradable chitosan so that the nanoparticles possessed positive surface charge, high siRNA loading, high transfection efficiency and low toxicity. It was found that the nanoparticle diameter and positive zeta potential increase as the chitosan coating concentration increases. Chitosan-modified PLGA nanoparticles showed excellent siRNA binding ability and effective protection of oligonucleotides from RNase degradation. Small interfering RNA-loaded nanoparticles were successfully delivered into the HEK 293T cell line, and the silencing of GFP expression was observed. In addition, the cytotoxicity assay revealed that nanoparticles had relatively low cytotoxicity [22]. Chitosan surface-modified PLGA nanospheres were also prepared by an emulsion solvent diffusion (ESD) method. Chitosan-modified PLGA nanoparticles showed much higher encapsulation efficiency than unmodified plain PLGA nanoparticles. Small interfering RNA-loaded chitosan-modified PLGA nanoparticles were taken up more effectively by the cells than plain PLGA nanoparticles. The gene silencing efficiency of modified PLGA nanoparticles was higher and more prolonged than those of plain PLGA nanoparticles and naked siRNA [23]. Chitosan-modified PLGA nanoparticles loaded with TF siRNA was used as a new external stent prepared by hybrid ultrafine fibrous membrane as a therapy for vein graft disease; because of the introduction of chitosan, which is a naturally hydrophilic polymer, the hybrid membranes showed good water absorption properties. It was found that the external stent prepared by chitosan-PLGA nanoparticles inhibits early neointima formation in rat vein grafts [24].

2.2 Chitosan nanoparticles

Chitosan is a naturally occurring biocompatible, biodegradable polycation with low toxicity, which is a very promising carrier for delivering siRNA. Owing to the interaction of anionic siRNA and cation chitosan, siRNA can be complexed and loaded into chitosan nanoparticles, as shown in **Figure 3**. Chitosan has been used successfully to form complex with siRNA against GFP. The spherical and stable chitosan-siRNA nanoparticles with 83 – 94% complex efficiency can be formulated under mild electrostatic interaction. Nearly 80% gene silencing efficiency of chitosan-siRNA nanoparticles was achieved [25].

Small interfering RNAs have been loaded in chitosan nanoparticles for cancer therapy with encouraging results [26].

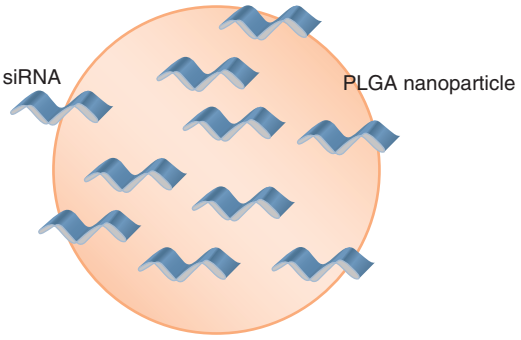

Figure 2. The biodegradable polymeric PLGA nanoparticles loaded with siRNA.
PLGA: Poly(DL-lactide-*co*-glycolide); siRNA: Small interfering RNA.

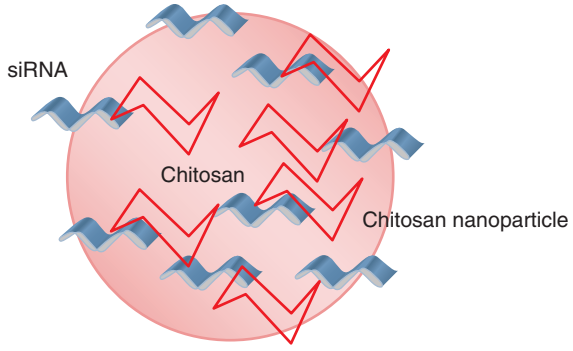

Figure 3. The chitosan nanoparticles showing interaction of siRNA with cationic chitosan molecules.
siRNA: Small interfering RNA.

Chitosan nanoparticle-mediated delivery of a shRNA-expressing vector has been used to inhibit TGFB1 expression in the human rhabdomyosarcoma cell line RD. Knockdown of TGFB1 by shRNA resulted in a decrease in RD cell growth *in vitro* and tumorigenicity in nude mice [27]. FHL2 siRNA formulated within chitosan nanoparticles could knock down ~ 69.6% FHL2 gene expression, which is very similar to the 68.8% reduced gene expression when siRNA was transfected with the liposome lipofectamine. The blocking FHL2 expression by siRNA could also inhibit the growth and proliferation of human colorectal cancer Lovo cells [28]. Twenty-nanometer-diameter chitosan nanoparticles could be prepared for siRNA delivery using an ionic gelation method, using sodium tripolyphosphate as a crosslinker. It was observed that complexes induced significant transfection in neuronal Neuro2a cells [29]. In addition, chitosan nanoparticles can be used for efficient delivery of siRNA into the neuronal tissues *in vivo* [30]. Core-shell poly(alkyl cyanoacrylate) chitosan nanoparticles have been prepared for intravenous administration of chemically unmodified siRNA orientated towards the junction oncogene of the papillary thyroid carcinoma. *In vivo*, the antisense siRNAs associated with the nanoparticles lead to a strong antitumoral activity. The tumor growth was almost stopped after intravenous injection of nanoparticles, whereas in all control experiments the tumor size increased at least 10 times [31]. Chitosan nanoparticles were labeled with Arg-Gly-Asp (RGD) peptide as a tumor-targeted delivery system for siRNA. RGD-labeled nanoparticles significantly increased selective intratumoral delivery in orthotopic animal models of ovarian cancer. Targeted silencing of multiple growth promoting genes (*POSTN*, *FAK* and *PLXDC1*) along with therapeutic efficacy in the SKOV3ip1, HeyA8 and A2780 models can be achieved using siRNA-loaded RGD-labeled chitosan nanoparticles. *In vivo* tumor vascular targeting was achieved using the RGD-labeled nanoparticles by delivering PLXDC1-targeted siRNA into the $\alpha_v\beta_3$ integrin-positive tumor endothelial cells in the A2780 tumor-bearing mice [32].

The mucoadhesive and mucopermeable properties of the cationic polysaccharide chitosan were utilized to deliver siRNA across mucosal epithelium and provided a platform for targeting human diseases with RNAi therapeutics [33]. Imidazole-modified chitosan/siRNA nanoparticles were used to mediate gene silencing after administration by means of either the intravenous (i.v.) or the intranasal (i.n.) routes. PEGylated nanoparticles for i.v. delivery demonstrated significant knockdown of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) enzyme in both lung and liver at as low as 1 mg/kg siRNA dose. The dose-dependent silencing of apolipoprotein B in the liver was also shown. Significant silencing of GAPDH protein expression was seen in the lungs with only 0.5 mg/(kg day). Small interfering RNA was delivered over three consecutive days via i.n. delivery [34].

Chitosan-complexed siRNA nanoparticles prepared by incorporation of locked nucleic acid (LNA) can achieve gene silencing in the bronchoepithelium of mice lungs following intranasal delivery and improved serum stability and reduced off-targeting effects *in vitro*. The naked siLNA administered intravenously efficiently reduces EGFP protein expression. A similar effect is obtained with intranasal delivery of chitosan nanoparticles containing siLNA, whereas intranasally instilled naked siLNA did not achieve a knockdown [35]. Normally, naked siRNA showed rapid renal clearance, with circulatory half-life < 5 min; but it could be extended to > 30 min by chitosan and cholesterol conjugation. Intact chemically modified siRNA could be detected in several organs at 30 min but disappeared at 24 h, except for heavy LNA-modified and cholesterol-conjugated siRNA in the lungs. High siRNA accumulation of the chitosan/siRNA nanoparticles within the kidney was observed 24 h post-administration [36].

Secretion of TNF-α by macrophages plays a predominant role in the development and progression of rheumatoid arthritis. The knockdown of TNF-α expression in systemic macrophages achieved by intraperitoneal (i.p.) administration

of chitosan/siRNA nanoparticles in mice downregulated systemic and local inflammation, thereby presenting a new strategy for arthritis treatment [37].

Chitosan and trimethylchitosan (TMC)-siRNA nanoparticles were produced by a simple complexation technique or by ionic gelation using tripolyphosphate (TPP). TMC-siRNA nanoparticles were stable in physiological condition and cellular uptake was increased compared with chitosan polyplexes. However, improvement of transfection efficiency was low regarding cellular uptake of these complexes [38]. To improve the endosomal escape of siRNA from chitosan nanoparticles, pH-sensitive methacrylic acid (MAA) copolymer was added to TMC-siRNA formulations. A swelling behavior resulting from a decrease in pH was observed and was found to be dependent on MAA content in the complexes. MAA-TMC-siRNA complexes were able to transfect L929 cells with greater efficiency than corresponding TMC-siRNA complexes [39]. Chitosan nanoparticles encapsulating siRNA were also prepared using a coacervation method in the presence of polyguluronate (PG), which is isolated from alginate and is strongly related to ionic interactions of negatively charged alginate. Nanoparticles showed low cytotoxicity and were useful in delivering siRNA to HEK 293FT and HeLa cells [40].

2.3 Dextran and cyclodextrin nanoparticles

Biocompatible, lipid-modified dextran nanoparticles were used as the platform for multi-drug resistance MDR1 siRNA delivery. Multi-drug-resistant osteosarcoma cell lines (KHOS (R2) and U-2OS(R2)) were treated with the MDR1 siRNA nanoparticles. Combination therapy of the MDR1 siRNA-loaded nanoparticles with increasing concentrations of doxorubicin was analyzed. MDR1 siRNA-loaded dextran nanoparticles efficiently suppress P-gp expression in the drug-resistant osteosarcoma cell lines. This approach may be capable of reversing drug resistance by increasing the amount of drug accumulation in MDR cell lines [41].

A targeted nanoparticle formulation of siRNA, denoted as CALAA-01, has been developed for the first in-human Phase I clinical trial. The nanoparticle system consists of a cyclodextrin-containing polymer, a PEG steric stabilization agent, and human transferrin (Tf) as a targeting ligand for binding to transferrin receptors (TfR) that are typically upregulated on cancer cells. The four-component formulation is self-assembled into nanoparticles in the pharmacy and administered intravenously to patients [42]. Tumor biopsies from melanoma patients obtained after treatment show the presence of intracellularly localized nanoparticles in amounts that correlate with dose levels of the nanoparticles administered. Furthermore, a reduction was found in both the specific messenger RNA (M2 subunit of ribonucleotide reductase (RRM2)) and the protein (RRM2) levels when compared with pre-dosing tissue. It was demonstrated that siRNA administered systemically to a human can produce a specific gene inhibition by an RNAi mechanism of action [43].

2.4 Cationic polyplex

Cationic polymers are widely investigated as siRNA carriers. Cationic polymers and siRNA self-assemble into siRNA polyplexes owing to electrostatic interaction, which has been shown to silence genes (**Figure 4**). Polyplexes can also be used to modulate pharmacokinetics and intracellular trafficking to improve the therapeutic efficacy of RNAi-based therapeutics. The inclusion of functional components into the nanoparticles can control cellular trafficking and RNA release [44]. Fluorescence fluctuation spectroscopy has been used to monitor quantitatively the disassembly of siRNA containing polyplex in full serum. Gene silencing efficacy of siRNA polyplexes in serum-containing media can be explained very well by their disassembling behavior in these media. [45].

Polyethylenimine, a widely used cationic polymeric vector with high transfection efficiency, has been used widely for gene delivery and RNAi. It was reported that linear PEI-based nanoparticles encapsulating siRNA were preferentially and avidly engulfed by regulatory dendritic cells (DCs) expressing CD11c and programmed cell death 1 ligand 1 (PD-L1) at ovarian cancer locations in mice. The intrinsic TLR5 and TLR7 stimulation of siRNA-PEI polyplex synergizes with the gene-specific silencing activity of siRNA to transform tumor-infiltrating regulatory DCs into DCs capable of promoting therapeutic antitumor immunity [46]. PEI could be converted into nanoparticles by introducing ionic and covalent crosslinkers with varying proportion of 1,6-hexanebisphosphate, adipic acid and 1,4-butane dialdehyde to obtain nanoparticles, respectively. Adipic acid-PEI nanoparticle/DNA complex showed higher transfection efficiency (1.5 – 7.8-fold) than the native PEI (25 kDa) and some commercially available transfection reagents with cell viability > 85%. Transfection with GFP-siRNA inhibited expression of transfected *GFP* gene by ~ 81 – 92%. Intravenous delivery of ^{99}Tc-labeled 1,4-butane dialdehyde-PEI/DNA complex to female Balb/c mice revealed the presence of the complex in most of the organs, with the highest retention in liver [47]. PEI and siRNA nanocomplex was also used to study the effect of PEI on respiratory tissue and transfection efficiency of siRNA. It was found that PEIs represent powerful siRNA delivery tools with reduced cytotoxicity and minor pro-inflammatory potency. However, in relation to response levels observed on crystalline silica exposures, some PEI-induced proapoptotic and pro-inflammatory responses might not be considered completely harmless [48]. Nanoparticles based on PEI modified with stearic acid (StA) were used to deliver siRNA for efficient signal transducer and activator of transcription 3 (STAT3), which has been shown to impart several oncogenic features in many solid and blood tumors, and downregulation in B16 melanoma cells. Compared with the PEI complexes, the PEI-StA complexes showed higher potency in STAT3 silencing in B16 cells accompanied by a significant induction of IL-6 secretion and a reduction of VEGF production. *In vivo* results indicated significant

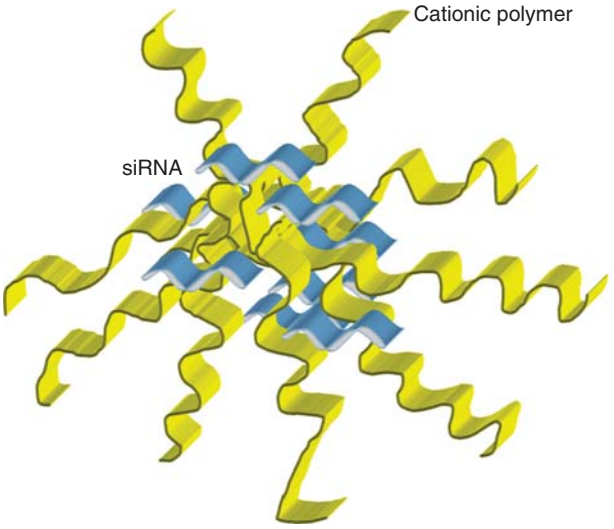

Figure 4. The formation of polyplex by interaction of siRNA with polycationic polymers.
siRNA: Small interfering RNA.

regression in tumor growth and tumor weight after siRNA/PEI-StA treatment as compared with the siRNA/PEI polyplex [49].

PEI nanoparticles were also prepared by acylating PEI with propionic anhydride followed by crosslinking with polyethylene glycol-bis(phosphate). The nanoparticles' size was found to be ~ 110 nm. For electro-neutralization of negative charge of siRNA a higher amount of nanoparticles was required as compared with native PEI. The gene silencing efficiency of PEI nanoparticles was found to be comparable to commercially available transfecting agent lipofectin but with reduced cytotoxicity [50]. Branched PEI (25 kDa) was ionically interacted with alginic acid (Al) to block different proportions (2.6 – 5.7%) of amines in PEI to form a series of nanocomposites, PEI-Al. These nanocomposites, on interaction with DNA, protected it against DNase I. The PEI-Al nanoparticles were nearly non-toxic to cells *in vitro*. The concentration of PEI-Al(4.8%) needed to deliver GFP-specific siRNA in COS-1 cells was 20 times lower than PEI (750 kDa)-Al (6.26%) [51]. PEI was used to interact ionically with hexametaphosphate (HMP), a compact molecule with high anionic charge density, to obtain nanoparticles (PEI-HMP). *In vitro* transfection efficiency of PEI-HMP (7.7%) was ~ 1.3 – 6.4-fold higher than that of the commercial reagents GenePORTER 2, Fugene and Superfect. PEI-HMP (7.7%) delivered GFP-specific siRNA in culture cells leading to > 80% suppression in *GFP* gene expression. A time course uptake of PEI-HMP (7.7%) nanoparticles showed the internalization of nanoparticles inside the cell nucleus in 2 h with negligible cytotoxicity [52].

Large macromolecules of polymers, such as PEI, often lead to the accumulation of toxicity and narrow therapeutic windows. An alternative approach is to use low-molecular-mass polymer, such as oligoethylenimine (OEI) 800 Da, which can be hydrophobically modified through the Michael addition of different alkyl acrylates. An optimal structure containing 10 hexyl acrylate residues per 1 OEI chain (OEI-HA-10) was found to be a promising candidate for siRNA delivery. A remarkable increase of biocompatibility without loss of efficiency could be achieved by coformulation of OEI-HA-10 with lauryl acrylate-modified OEI-LA-5 [53]. Bioreducible cationic polymer poly(cystaminebisacrylamide-diaminohexane) (poly(CBA-DAH)) containing repeated disulfide linkages on the polymer backbone was synthesized through Michael-type polyadditions of CBA to DAH monomers. Poly(CBA-DAH) could spontaneously form nanoscale polyelectrolyte complexes through electrostatic interactions with siRNA in an aqueous phase. These polyplexes were rapidly degraded under the reductive cytoplasmic environment, subsequently releasing the siRNA cargo into the cytoplasm where RNAi takes place, as a result of the breakdown of disulfide bonds in the polymers [54].

2.5 PEI-modified polymeric nanoparticles

Some nanoparticles were negatively charged owing to the nature of the composed polymers. Therefore, cationic PEI is sometimes used to convert the particles to positive charge. PEI was incorporated in the PLGA matrix to improve siRNA encapsulation and transfection efficiency in PLGA nanoparticles (**Figure 5**). PLGA-PEI nanoparticles were formulated using a double-emulsion solvent evaporation technique. The presence of PEI in PLGA nanoparticle matrix increased siRNA encapsulation about twofold and also resulted in twofold higher cellular uptake of nanoparticles. Serum stability and lack of cytotoxicity further add to the potential of PLGA-PEI nanoparticles in gene silencing-based therapeutic applications [55]. In another study, PEI was incorporated into the PLGA particles by a spontaneous modified emulsification diffusion method. Incorporation of PEI into PLGA particles with the PLGA-to-PEI weight ratio 29:1 was found to produce spherical and positively charged nanoparticles. Particle size of ~ 100 nm was obtained when 5% (m/v) PVA was used as a stabilizer. *In vitro* cell culture studies subsequently revealed that PLGA-PEI nanoparticles with adsorbed siRNA could efficiently silence the targeted gene in mammalian cells, better than PEI alone, with acceptable cell viability [56].

Biodegradable nanoparticles composed of polycaprolactone (PCL) and PEI were successfully synthesized for the delivery of sh/siRNA in lung cancer cells. PCL-PEI nanoparticles efficiently and safely delivered siRNA in lung cancer cells. The delivered Akt1 siRNA silenced Akt1 protein and reduced the cancer cell survival, proliferation, malignancy and metastasis [57]. Poly(ethylene oxide)-modified poly(beta-amino ester) (PEO-PbAE) and PEO-modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticles were formulated to encapsulate efficiently MDR-1 silencing siRNA and

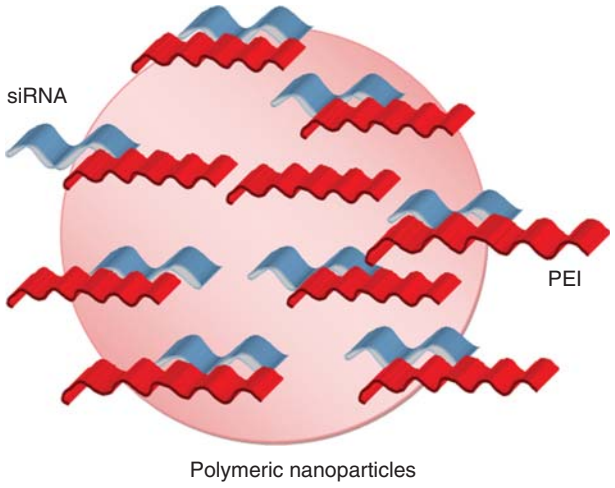

Figure 5. The polymeric nanoparticles modified with cationic PEI and loaded with siRNA.
PEI: Polyethyleneimine; siRNA: Small interfering RNA.

paclitaxel, respectively. On administration in multi-drug-resistant SKOV3(TR) human ovarian adenocarcinoma cells, siRNA-mediated MDR-1 gene silencing was evident at 100 nM dose. Combination of MDR-1 gene silencing and nanoparticle-mediated delivery significantly influenced the cytotoxic activity of paclitaxel in SKOV3(TR) cells, similar to what was observed in drug-sensitive SKOV3 cells [58].

To overcome instability and low transfection efficiency, PEG-PEI was synthesized and investigated as a non-viral carrier of siRNA targeting CD44v6 in gastric carcinoma cells. The transfection efficiency of PEG-PEI/siRNA at N/P ratio of 15 was ~ 72.5%, which was higher than that observed using Lipofectamine 2000 and PEI as delivery carriers. Cytotoxicity of PEG-PEI was lower than that of PEI. When N/P was < 15, PEG-PEI/siRNA was less toxic than Lipofectamine 2000/siRNA. RT-PCR and western blot analyses of CD44v6 expression demonstrated the gene silencing effect of PEG-PEI/siRNA at N/P 15 [59].

Both cationic polyplex and PEI-modified nanoparticles have polycationic charge on the surface of polymers and particles, and usually bring cytoxicity when delivering siRNA or pDNA. Therefore, it is critical to optimize these delivery systems in order to minimize their toxicity before they can be used clinically.

2.6 Lipid nanoparticle and lipoplex

Lipid nanoparticles, usually composed of different compositions of cationic lipids, PEG and cholesterol, are also used to deliver siRNA and pDNA. Lipid-based nanoparticle technology has developed from chemical drug carrier into an efficient multifunctional siRNA tumor-targeting delivery system [60]. A lipid nanoparticle system with luciferase siRNA was developed and measured by *in vivo* bioluminescence imaging, which correlated well with the results from parallel *ex vivo* analyses of luciferase mRNA and protein levels in the liver. RNAi-mediated target silencing was further confirmed by the detection of RNAi-specific target mRNA cleavage. With identical components, lipid nanoparticles containing 2% PEG are more potent than those with 5.4% PEG [61].

It was reported that HER-2 siRNA could suppress the growth of human nasopharyngeal KB tumor xenografts by intratumoral injection with lipid-based nanoparticles. Antitumor activity can be enhanced by RNAi in combination with paclitaxel (PTX) using HER-2 siRNA and HER-2 shRNA-expressing pDNA (HER-2 shRNA pDNA). Suppression of HER-2 expression by siRNA or shRNA pDNA caused significant reduction of proliferation by inducing apoptosis and enhancing the sensitivity for PTX in HER-2-positive KB cells. HER-2 shRNA pDNA plus PTX largely extended the mean survival time compared with HER-2 siRNA plus PTX [62]. Cationic lipid nanoparticles composed of cholesteryl-3beta-carboxyamidoethylene-*N*-hydroxyethylamine (OH-Chol) and Tween 80 have been developed and evaluated for the transfection efficiencies of pDNA and siRNA into human prostate tumor PC-3 xenografts. Lipid nanoparticles showed effective transfection of pDNA and siRNA when directly injected into the xenografts. For targeted delivery to tumors, vitamin folic acid has been utilized for folate receptor (FR)-mediated drug delivery because FR is frequently overexpressed on many types of human tumor. Folate-linked nanoparticles composed of OH-Chol, Tween 80 and folate-poly (ethylene glycol)-distearoylphosphatidylethanolamine conjugate was synthesized. Tumor growth of FR-positive human nasopharyngeal tumor KB xenografts was significantly inhibited when nanoparticle with a therapeutic gene was injected intratumorally [63]. Cisplatin nanoparticles, nanoprecipitates of cisplatin encapsulated in phospholipid bilayers, show increased *in vitro* toxicity compared with the free drug towards a panel of human ovarian carcinoma cell lines. The high cytotoxicity of cisplatin nanocapsules requires caveolin-1-dependent endocytosis that is followed by release of the drug from a late endosomal/lysosomal compartment and cisplatin-DNA-adduct formation [64].

Octaarginine (R8)-modified lipid envelope-type nanoparticles were reported for siRNA delivery (R8-MEND). A successful endosomal escape was achieved by using a pH-dependent fusogenic peptide (GALA) modified on a lipid mixture that was optimized for endosomal fusion. Gene knockdown of the suppressor of cytokine signaling 1, a negative-feedback regulator of the immune response in primary mouse bone marrow-derived dendritic cells, resulted in an enhanced phosphorylation of signal transducers and activators of transcription factor 1 (STAT1) and the production of pro-inflammatory cytokines. Consequently, siRNA loaded in R8/GALA-MEND enhances dendritic cell-based vaccine potency *in vivo* [65]. MEND can be modified further with polyethylene glycol into PEG-peptide-DOPE MEND (PPD-MEND) for *in vivo* delivery to tumor tissue.

PPD-MEND can be modified further with a pH-sensitive fusogenic GALA peptide (GALA/PPD-MEND). It was demonstrated that the introduction of both of a pH-sensitive fusogenic GALA peptide and PPD into the MEND facilitates endosomal escape, thereby enhancing the efficiency of siRNA delivery and gene silencing [66].

Small interfering RNA or DNA can form electrostatic complex with cationic lipid, and the new complex is called lipoplex, which can be used as a non-viral nucleic acid delivery system. A panel of new nitrogen heterocycle cholesteryl derivatives containing a biodegradable carbamate linker was synthesized. Optimally acting piperazine and cyclen compounds had nucleic acid-binding and lipoplex properties that were suitable for their use as non-viral vectors. It was found that the cyclen-containing compound possessing two cholesteryl moieties resulted in efficient siRNA-mediated target gene silencing but was a poor reagent for DNA transfection [67]. Five long-chain *N*(1),*N*(12)-diacyl lipopolyamines: *N*(1),*N*(12)-[didecanoyl, dimyristoyl, dimyristoleoyl, distearoyl and dioleoyl]-1,12-diamino-4,9-diazadodecane, were synthesized from the naturally occurring polyamine spermine. By incorporating two long aliphatic chains and varying their acylation position, length and oxidation state in a stepwise manner, efficient siRNA formulation and delivery to primary skin and cancer cell lines was reported. Adding two C20 or C22 chains, both mono-*cis*-unsaturated, *N*4,*N*9-dieicosenoyl spermine and *N*4,*N*9-dierucoyl spermine gave efficient siRNA delivery vectors, even in the presence of serum, and with excellent cell viability [68,69]. Synthetic fluorinated lipospermines were used to bind DNA and siRNA and the transfection efficiency and toxicity of the resulting lipoplexes in cell lines were evaluated. Three lipopolyamines displaying fluorous tags close to their cationic polar head ('HFP' polyamines) were synthesized. It was found that lipospermines displaying fluorous tags close to their cationic polar head bind to and deliver pDNA and siRNA with high cell viability in different cell lines [70].

RNAi has generated significant interest as a strategy to suppress viral infection, but in some cases the antiviral activity of unmodified siRNA has been attributed to activation of innate immune responses. Lipidoid 98N12-5(1) formulated with unmodified siRNA targeting the influenza nucleoprotein gene showed antiviral activity. It was demonstrated that innate immune activation caused by unmodified siRNA can have therapeutically relevant effects, and that these non-RNAi effects can be controlled through chemical modifications and drug delivery [71]. Cationic lipid *N*′,*N*′-dioctadecyl-*N*-4,8-diaza-10-aminodecanoylglycine amide was synthesized and formed lipoplexes with siRNA that are able to mediate the functional delivery of two anti-hepatitis B virus (HBV)-siRNAs to murine liver *in vivo* with minimal observable liver toxicity and immune stimulation. Specific knockdown of HBV infection parameters (virion and hepatic mRNA levels) is observed that is at least equivalent to the impact of extensive treatment with lamivudine [72].

To facilitate the escape of lipoplex from endosomes, a particular class of carriers including the polymers, peptides and lipids was developed based on exploitation of the imidazole ring as an endosome destabilization device to favor the nucleic acids' delivery in the cytosol. The imidazole ring of histidine is a weak base that has the ability to acquire a cationic charge when the pH of the environment drops below six. As has been demonstrated for polyhistidine, this phenomenon can induce membrane fusion and/or membrane permeation in an acidic medium. It was also found that the accumulation of histidine residues inside acidic vesicles can induce a proton sponge effect, which increases their osmolarity and swelling. In addition, histidylated carriers are often weakly cytotoxic, making them promising chemical vectors for nucleic acid delivery [73].

A cationic lipopeptide, gemini-like amphiphilic peptides or 'geminoids', was synthesized for siRNA and pDNA delivery. The SPKR peptide, inspired by biological nucleic acid binding motifs, was appended with unsaturated (oleoyl/oleyl) alkyl tails. The compound showed remarkable DNA and siRNA delivery, without lysogenic helper lipid, in a variety of cells, with a moderate cytotoxic effect. It aggregates to nanoparticles that combine with DNA to lipoplexes, which undergo a change from lamellar to the more lysogenic hexagonal packing on lowering the pH [74].

Like the cationic polyplex mentioned earlier, it is critically important to minimize the toxicity associated with the lipoplex delivery system in order to develop clinically viable delivery systems for siRNA.

2.7 Liposome

Inspired by progress with lipid-based systems in drug delivery, efforts have been dedicated to the development of liposomal siRNA delivery systems. Many of the lipid-based delivery vehicles self-assemble with siRNA through electrostatic interactions with charged amines, generating multilamellar lipoplexes with positively charged lipid bilayers separated from one another by sheets of negatively charged siRNA strands, therefore forming liposomes. As siRNA is hydrophilic, it can also be incorporated inside the core of liposome, as shown in **Figure 6**. Internalization of liposome into cells typically occurs through endocytosis and release of siRNA or pDNA through endosomal escape. The size of the liposome is important as carriers < 100 nm in diameter have been reported to have higher accumulation levels in tumors, hepatocytes and inflamed tissue, whereas larger particles tend to be taken up by Kupffer cells or other components of the reticuloendothelial system (RES). To reduce RES uptake and increase circulation time, carriers have been modified on the surface with hydrophilic materials, such as PEG [75]. P-selectin was covalently attached to the surface of nanoscale liposomes to create targeting nanoparticles. Small interfering RNA was encapsulated by these nanoscale liposomes, and the liposomes were stabilized by PEGylation with

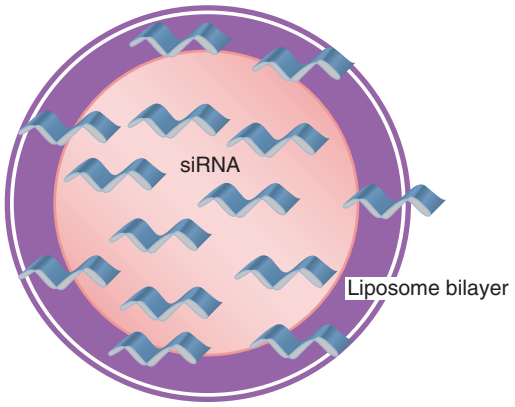

Figure 6. Liposome loaded with siRNA inside the hydrophilic core and intercalated in a bilayer.
siRNA: Small interfering RNA.

1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide (polyethylene glycol)-2000] (DSPE-PEG2000). The coated surface could specifically capture targeted cells from physiological shear flow, efficiently deliver encapsulated siRNA into adherent cells and dramatically silence the targeted gene neutrophil elastase. A high localized concentration of siRNA was achieved in the circulatory system, providing circulating target cells adequate time to interact with therapeutic materials [76]. Cationic liposome was developed for systemic delivery of raf ASO (LErafAON) and raf siRNA (LErafsiRNA) to human tumor xenografts grown in athymic mice. The cationic liposomes were prepared using dimyristoyl 1,2-diacyl-3-trimethylammonium-propane (DMTAP), phosphatidylcholine (PC) and cholesterol (CHOL). It was demonstrated that md-LErafAON could be systemically delivered and is a well-tolerated antisense therapeutic suitable for clinical evaluation [77]. Theoretically, cationic liposome is more effective than neutral liposome. However, it was reported that the use of neutral 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine-based nanoliposomes in murine tumor models was safe and 10- and 30-fold more effective than cationic liposomes and naked siRNA, respectively [78].

A PEGylated liposome-polycation-DNA nanoparticle (LPD) was designed for systemic, specific and efficient delivery of siRNA into solid tumors in mice by modification with aspargine-glycine-arginine (NGR) peptide, targeting aminopeptidase N (CD13) expressed in the tumor cells or tumor vascular endothelium. The dynamic light scattering data showed that LPD had improved stability compared with cationic liposomes after incubation with a high concentration of DSPE-PEG(2000), which is known to disrupt the bilayer [79]. LPD and anionic liposome-polycation-DNA (LPD-II) were developed for systemic co-delivery of doxorubicin and a therapeutic siRNA to MDR tumors. Three daily intravenous injections of therapeutic siRNA and doxorubicin (1.2 mg/kg) coformulated in either LPD or LPD-II nanoparticles showed a significant improvement in tumor growth inhibition [80]. Liposome-polycation-hyaluronic acid (LPH) nanoparticle formulation was developed and modified with tumor-targeting single-chain antibody fragment (scFv) for systemic delivery of siRNA and microRNA (miRNA) into experimental lung metastasis of murine B16F10 melanoma. The siRNAs delivered by the scFv-targeted nanoparticles efficiently downregulated the target genes (*c-Myc*/*MDM2*/*VEGF*) in the lung metastasis [81].

A systemically injectable siRNA vehicle, the 'wrapsome', was developed recently, which contains siRNA and a cationic lipofection complex in a core that is fully enveloped by a neutral lipid bilayer and hydrophilic polymers. Wrapsome protected siRNA from enzymatic digestion, providing a long half-life in the systemic circulation. Moreover, siRNA wrapsome leaked from blood vessels within tumors into the tumor tissue, where it accumulated and was subsequently transfected into the tumor cells. It was found that siRNA wrapsome can be used to knockdown specific genes within tumors and thereby exert therapeutic effects against cancers [82]. Recently, a liver-specific siRNA delivery technology was developed using DTC-Apo composed of cationic liposomes (DTC) and apolipoprotein A-I (apoA-I). Post-administration of DTC-Apo/HCV-specific siRNA at a dose of 2 mg siRNA/kg inhibited viral gene expression by 65 – 75% in the liver on day 2. Improved activity (95% knockdown on day 2) without immunotoxicity was obtained by 2′-OMe-modification at two U sequences on its sense strand. The gene silencing effect of the modified siRNA was still maintained at day 6, whereas the unmodified one lost RNAi activity after day 4 [83]. Vaginal instillation of siRNA using liposomes has led to silencing of endogenous genes in the genital tract and protection against challenge from infectious disease. Nanoparticles composed entirely of FDA-approved materials were used to render effective for gene silencing and knockdown of gene expression, which was observed proximal (in the vaginal lumen) and distal (in the uterine horns) to the site of topical delivery [84].

2.8 Dendrimers

The use of dendrimers for biomedical applications has had promising results lately. Dendrimers have been investigated for siRNA and antisense oligonucleotide delivery resulting from the interaction of positively charged dendrimer branches with siRNA, as shown in **Figure 7** [85]. Different generations of poly(amidoamine) (PAMAM) dendrimers have been used to bind and deliver siRNA. The molecular flexibility of PAMAMs plays a crucial role in the binding event, which is controlled by modulation between enthalpy and entropy of binding. Importantly, the ability of dendrimers to adapt to siRNA is strongly dependent on the generation and on the pH owing to backfolding. Whereas G4 (fourth generation) demonstrates good adaptability to siRNA, G6 behaves like a rigid sphere with a consistent loss in the binding affinity. G5 shows a hybrid behavior, maintaining rigid and flexible aspects, with a strong dependence of its properties on the

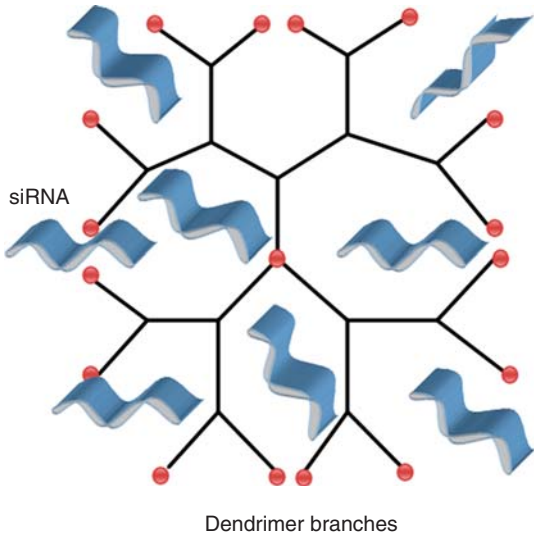

Figure 7. Dendrimer loaded with siRNA showing interaction of siRNA with cationic dendrimer branches.
siRNA: Small interfering RNA.

pH [86]. Cancer cell receptor-targeted internally quaternized and surface neutral PAMAM G4 dendrimer as well as PAMAM-paclitaxel conjugate were synthesized. Those dendrimers provide internal cationic charges for complexation with siRNA or antisense oligonucleotides and protection from degradation in systemic circulation. They can be efficiently internalized by cancer cells and preferentially accumulated in the tumor, resulting in prevention of adverse side effects of chemotherapy. In addition, their neutral-modified surface has low cytotoxicity [87]. In a similar study, the dendrimer contained a synthetic analogue of luteinizing hormone-releasing hormone (LHRH) as cancer targeting moiety. Only the targeted dendrimer-siRNA complex was able to decrease substantially the expression of a targeted *BCL2* gene, the untargeted complex could not [88]. PAMAM dendrimers were reacted with acetic anhydride to obtain controlled extents of primary amine acetylation, and then were complexed with siRNA. Dendrimers with up to 60% of primary amines acetylated formed ~ 200 nm complexes with siRNA. Approximately 20% of primary amines of PAMAM can be modified while maintaining the siRNA delivery efficiency of unmodified PAMAM, but higher degrees of amine neutralization reduced the gene silencing efficiency [89]. A folate receptor-targeted dendrimer, PEG-G3-(Gd-DTPA)11-(folate)5, was synthesized to detect FR-positive tumors, by using dynamic contrast-enhanced MRI. Green fluorescence was found in the KB cells in the cellular uptake experiment, but was not seen in other settings. The targeting ability of FR dendrimer was demonstrated both *in vitro* and *in vivo* [90].

Complexes can be formed between ethylendiamine (EDA) core PAMAM dendrimers and siRNA as a function of three variables: the ionic strength of the medium (lacking or containing NaCl), the dendrimer generation (G4, G5, G6 and G7) and the N/P ratio (nitrogen amines in dendrimer/phosphate in siRNA). It was showed that modifying the chemical structure of dendrimer is not the only way of achieving complex suitable for silencing activity. The simple use of a low ionic strength preparation medium has been critical to getting a small complex that can be captured by cells and, in particular, siRNA-G7, but not those formed by lower generation dendrimer, possessing structural constraints other than size that could favor its silencing activity [91]. Structurally flexible triethanolamine (TEA) core PAMAM dendrimers were synthesized to deliver an Hsp27 siRNA effectively into prostate cancer (PC-3) cells by forming stable nanoparticles with siRNA, protecting siRNA nanoparticles from enzymatic degradation and enhancing cellular uptake of siRNA. Silencing of the *hsp27* gene by low toxicity dendrimer complex led to induction of caspase-3/7-dependent apoptosis and inhibition of PC-3 cell growth *in vitro* [92].

Poly(propylene imine) (PPI) dendrimers were also used to formulate siRNA nanoparticles. To provide lateral and steric stability to withstand the aggressive environment in the bloodstream, the formed siRNA nanoparticles were caged with a dithiol containing crosslinker molecules followed by coating them with PEG polymer. A synthetic analogue of LHRH peptide was conjugated to the distal end of PEG polymer to direct the siRNA nanoparticles specifically to the cancer cells. *In vivo* body distribution data confirmed high specificity of the proposed targeting delivery approach, which prevents adverse side effects of the treatment on healthy organs [93].

Carbosilane dendrimer was developed to deliver siRNA efficiently to postmitotic neurons to study the function of hypoxia-inducible factor-1-alpha during chemical hypoxia-mediated neurotoxicity. It was found that this type of vector is a good alternative to viral vectors for achieving very high transfection levels in neurons [94]. In another study, a carbosilane dendrimer with 16 positive charges per molecule was tested and found to be capable of binding and releasing siRNA and antisense oligonucleotides *in vitro*. Exposing macrophages to this dendrimer or dendriplex causes multiple gene expression changes, but no specific action of random siRNA was detected [95].

Dendrimer-conjugated magnetofluorescent nanoworms (dendriworms) were developed as a modular platform for siRNA delivery *in vivo*. This platform maximizes endosomal escape to produce robustly protein target knockdown *in vivo*, and is tolerated well in mouse brain. Small interfering RNA-carrying dendriworms can be readily internalized by cells and allow endosomal escape across a wide range of loading doses, whereas dendrimers or nanoworms alone are inefficient. Dendriworms carrying siRNA against EGFR reduce protein levels of EGFR in human glioblastoma cells by 70 – 80%, 2.5-fold more efficiently than commercial cationic lipids. Dendriworms were well tolerated after 7 days of

Table 1. Selected examples of clinical trials of siRNA by using nanoparticle delivery systems.

Company	Type of siRNA	Formulation	Delivery routes	Targeted disease	Clinical status
-	ApoB siRNA	SNALP/lipid nanoparticles	Intravenous	Hypercholesterolemia	Phase I [99]
Calando (Pasadena, CA, USA)	CALAA-01	Cyclodextrin nanoparticles with transferrin	Intravenous	Solid tumor/melanoma	Phase I [42,43,98]
Alnylam (Cambridge, MA, USA)	ALN-VSP02	Tekmira's lipid nanoparticle delivery system	Intravenous	Solid cancer	Phase I [100]
Marina Biotech (Bothell, WA, USA)	siRNA	tauRNAi/liposome	Intravenous	Hepatocellular carcinoma	Preclinical stage [100]
Marina Biotech (Bothell, WA, USA)	shRNA	tkRNAi/modified bacterial vectors	Oral	Familial adenomatous polyposis	Phase I [100]
Silence Therapeutics (London, UK)	Atu027 specific to *PKN3* gene	AtuPlex/liposome	Intravenous	Colorectal cancer metastasizing to the liver	Phase I [100]
Sirnaomics (Gaithersburg, MD, USA)	Two siRNAs targeting TGFBI and COX-2	STP705 nanoparticle encapsulation of two siRNAs	Intravenous	Wound healing	Phase I

ALN-VSP02: VEGF-A-specific siRNA; shRNA: Short hairpin RNA; siRNA: Small interfering RNA.

convection-enhanced delivery to the mouse brain and in an EGFR-driven transgenic model of glioblastoma [96]. PAMAM dendrimer and Tat peptides were conjugated to bacterial magnetic nanoparticles (BMPs) for the construction of an efficient and targeted gene delivery system with transmembrane ability for the gene therapy of brain tumors. Tat-BMPs-PAMAM was complexed with siRNA expression plasmid (psiRNA) corresponding to the open reading frame of the human *EGFR* gene (psiRNA-EGFR) to downregulate the *EGFR* gene by electrostatic interaction. It was found that Tat-BMPs-PAMAM, with its targeted delivery and transmembrane ability, may have potential applications in the targeted gene therapy of brain tumors [97].

3. Clinical trials of siRNA by nanoparticles

At present, there are a few clinical trials of siRNA therapeutics, including several formulations using nanoparticles, as shown in **Table 1**. CALAA-01, developed by Calando, is a nanoparticle (Rondel) delivery system composed of cyclodextrin, adamantine-modified stabilizer, adamantine-modified targeting ligand (transferrin) and siRNA. It was the first Phase I clinical trial in humans of siRNA in nanoparticles. In the patients who participated in the trial, CALAA-01 was well tolerated and no serious adverse effects were observed. Melanoma biopsies showed nanoparticles in the targeted cancer tissue [42,43,98]. Several other companies, including Tekmira, Alnylam, Marina, and so on, also have siRNA nanoparticle products in either the preclinical or clinical phases.

4. Conclusion

Since the discovery of the RNAi mechanism and siRNA as promising therapeutical agents, different non-viral nanoparticle carrier systems for siRNA delivery have been investigated extensively recently. The most important nanoparticle systems include biodegradable polymeric nanoparticles, polyplex, lipid nanoparticles, lipoplex, liposomes and dendrimers. The characteristics, components of preparations, and *in vitro* or *in vivo* delivery studies have been discussed for each system. Non-viral nanoparticle systems provide a very promising clinical strategy for delivering therapeutic siRNA for various diseases. At present, there are several exciting clinical studies of siRNA nanoparticle products.

5. Expert opinion

In the past few years since the Nobel Prize-winning RNAi technology revealed this new and exciting research arena, the field of drug delivery of siRNA has been developing very fast. Different non-viral nanoparticle systems, including biodegradable nanoparticles, polyplex, liposome, lipoplex, dendrimers and others, have been explored to deliver siRNA therapeutics *in vitro* and *in vivo*. So far, a few preliminary

clinical trials of nanoparticles have produced promising results. However, there is still much research that needs to be done in order to pave the way to successful clinical applications. The most important issues include, but are not limited to, encapsulation efficiency, formulation stability of siRNA, degradation in circulation, endosomal escape and delivery efficiency, targeting, toxicity and off-target effect. For example, the polymeric nanoparticles usually release siRNA at a slower rate in endosome, and their endosomal escape is not very efficient, which may result in lower transfection efficiency. The liposome is not thermodynamically stable, and has lower encapsulation efficiency and leaking problems. Lipoplex and polyplex utilize cationic lipids or polymers, sometimes bringing serious toxicity issues. Dendrimers also have similar problems and may not be biodegradable in the body. In addition, all of these nanoparticle delivery systems may be cleared very rapidly in the bloodstream owing to macrophages or the RES. Therefore, this problem also needs to be addressed. However, it will make delivery systems more complicated to prepare and more difficult to use clinically.

If RNAi technology can be harnessed successfully, it can offer a revolutionary therapeutical strategy for curing many diseases that are related to malicious genes. To achieve this goal, an efficient multifunctional drug carrier system, such as nanoparticles, must be developed before the siRNA can be used clinically. Besides developing effective delivery systems mentioned above, pharmacology and pharmacokinetics studies also present another great challenge for nanoparticle delivery systems as a result of the unique nature of siRNA oligonucleotides compared with small molecules.

The authors expect that this exciting field will develop very rapidly in the coming years. More new delivery systems will be developed in addition to the most important ones, which are discussed in this review. More siRNA delivery systems, perhaps not be limited to nanoparticle carrier systems, will move into the different phases of clinical trials, even though it is difficult to predict which ones will be successfully developed and eventually approved for use with patients. Considering safety issues of materials and the FDA approval process, biodegradable and biocompatible nanoparticle carrier systems for siRNA possess great potential to be approved first and therefore are of great interest among many researchers at present.

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Declaration of interest

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