Published in final edited form as:
Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010 ; 2(3): 305–315. doi:10.1002/wnan.81.

siRNA applications in nanomedicine

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Abstract

The ability to specifically silence genes using RNA interference (RNAi) has wide therapeutic applications for the treatment of disease or the augmentation of tissue formation. RNAi is the sequence-specific gene silencing mediated by a 21 to 25 nucleotide double stranded small interfering RNA (siRNA) molecule. siRNAs are incorporated into the RNAi-induced silencing complex (RISC), which mediates mRNA sequence specific binding and cleavage. Although RNAi has the potential to be a powerful therapeutic drug, its delivery remains a major limitation. The generation of nano-sized particles is being investigated to enhance the delivery of siRNA-based drugs. These nanoparticles are generally designed to overcome one or more of the barriers encountered by the siRNA when trafficked to the cytosol. In this review, we will discuss recent advances in the design of delivery strategies for siRNA, focusing our attention to those strategies that have had in vivo success or have introduced novel functionality that allowed enhanced intracellular trafficking and/or cellular targeting. The review will first discuss the different barriers that must be overcome for efficient siRNA delivery. Second we will discuss the approaches for siRNA delivery by size including direct modification of siRNAs (less than 10nm), self-assembled particles based on cationic polymers and cationic lipids (100 to 300 nm), neutral liposomes (< 200 nm), and macro scale matrices that contain naked siRNA or siRNA loaded nanoparticles (> 100 μm). Last, we will briefly discuss recent in vivo therapeutic successes.

Keywords

siRNA; Non-viral gene delivery; RNAi; siRNA nanoparticles; Nanomedicine

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There are currently three methods used to silence the expression of a target gene: antisense oligonucleotides (ODN), ribozymes, and RNA interference (RNAi). Until recently, ODNs had been the main hope for clinical applications. ODNs are short pieces of DNA or RNA complementary to messenger RNA (mRNA) sequences, which function by hybridizing with the mRNA creating a double stranded stretch. In the case of DNA ODNs, a RNA-DNA duplex is formed, which is recognized by an RNAse and degraded. This process has been successful in vitro, yet the lack of an appropriate delivery mechanism has made in vivo applications limited [1]. Ribozymes are catalytically active RNA composed for three helices which cleave single stranded regions of their own or other RNAs by trans-esterification or hydrolysis [1]. Due to their low stability in serum of only seconds to minutes, ribozymes have not been highly investigated for therapeutic applications. Consequently the use of siRNA for RNAi and gene silencing is quickly becoming the new paradigm for gene down

regulation with reports indicating siRNA is 1000-fold more effective than antisense ODNs in silencing target genes [2]. The process of RNAi was first described in worms in 1998 [1] and it is now believed to exist in all animals. The natural function of RNAi appears to be protection of the genome against invasion by mobile genetic material elements such as transposons and viruses, which produce aberrant RNA or dsRNA when they become active [3]. In lower organisms once this dsRNA (> 30nt) is inside the cell, the process of RNAi begins with the enzymatic degradation of the dsRNA into 21 nucleotide dsRNA segments that have 19 bases of complementary nucleotides and 3′ terminal overhangs of two nucleotides, termed small interfering RNA (siRNA) [3]. In the second stage of RNAi, siRNA along with nucleases and other proteins assemble into the RNA induced silencing complex (RISC) in the cytosol. The RISC complex enables the antisense strand of the siRNA to bind to the complementary sequence of mRNA, which in turn induces the degradation of the complementary mRNA (Figure 1). In mammalian cells only the second step of RNAi is possible without the activation of the interferon response [4].

Although other forms of siRNA exist, such as short hairpin RNA (shRNA) [5, 6], micro RNA (miRNA) [1, 5], and Piwi-interacting RNA (piRNA) [7], siRNA is the most commonly used for RNAi in therapeutic applications (Table 1). Conversely, shRNA is used more frequently in laboratory settings where permanent down regulation of a specific mRNA is desired, typically delivered using viral vectors [8]. Unlike siRNA, shRNA is delivered as a gene that encodes for the desired shRNA that when transcribed forms a hairpin structure recognized by a DICER type protein that generates the active shRNA. Therefore, the gene encoding for shRNAs has to enter the nucleus, which adds complexity to the delivery [5]. For this reason non-viral delivery of siRNA is viewed as more feasible than traditional non-viral delivery of plasmid DNA.

The use of siRNAs as therapeutics begins with (i) the design of siRNAs which target the mRNA of a protein that causes disease or whose down regulation results in tissue formation, (ii) the incorporation of siRNAs into a delivery vehicle that can efficiently deliver the siRNAs in vivo, and (iii) the delivery of the siRNA/vehicle complex to the desired tissue/site, where the siRNA can effectively silence the gene and achieve the desired therapeutic response. However, there are limitations with each of these steps. In this review, we will focus on the most recent advances in the delivery of siRNAs using non-viral approaches that have either shown in vivo efficacy or that utilize smart nanoparticles for delivery. We will also briefly mention strategies used to avoid off-target effects and provide a list of current therapeutic targets of siRNA delivery.

Limitations to siRNA delivery

The delivery of siRNA for biomedical applications is very promising due to its stringent sequence specific action as seen in recent successes in gene silencing both in vitro and in vivo [9, 10]. Yet, there are extracellular and intracellular limitations that must be addressed for the full therapeutic potential of siRNA to be realized. Table 2 summarizes the main limitations faced by siRNA through its delivery and internalization process.

siRNA stability

Major extracellular limitations to siRNA delivery include siRNA degradation, aggregation of siRNA nanoparticles in serum, and targeting. siRNAs, like most RNA molecules, are readily degraded by RNAses, which are ubiquitous both in the extracellular and the intracellular space. Naked siRNA in serum has been shown to have a half life of several minutes to an hour [4]. In order to stabilize siRNA in serum chemical modification has been used [1, 4, 11]. It was shown that modification of the sugars or backbone on siRNA by 2′-O-methyl and 2′-deoxy-2′-fluoro (OMe/F) or phosphorothioate linkages, respectively, resulted in enhanced resistance to nucleases and increased siRNA half-life [1, 4]. Polymer and/or lipid encapsulation strategies can also shield the vector against degradation, clearance, and an immune response.

Nanoparticle aggregation

Aggregation of siRNA nanoparticles typically occurs due to the surface charge of siRNA-loaded nanoparticles (typically net positive). Thus, the most common approach to prevent aggregation is to reduce the surface charge of the nanoparticles by introducing polyethylene glycol (PEG) [12] or sugar molecules (e.g. cyclodextrin) [13, 14], and hyaluronic acid [15]. Charge neutralization has the added benefits of increasing nanoparticle circulation time and allowing for nanoparticle targeting through limiting unspecific interactions between the positively charged nanoparticles and the negatively charged cell membrane.

Nanoparticle targeting

Targeting the appropriate tissue and/or cell is a major limitation for all nanoparticle based delivery strategies. Targeting has been introduced to siRNA containing nanoparticles through the modification of the nanoparticles with ligands or antibodies recognizing cell surface receptors or antigen integrins, respectively. Recent examples of ligands used to target siRNA containing nanoparticles in vivo include a monoclonal antibody-protamine fusion protein, which was able to selectively target leukocytes containing lymphocyte function-associated antigen-1 integrins [16], *N*-acetylgalactosamine, which was able to target hepatocytes [12], trasferrin, which was able to target tumor xenografts [13, 14], and RGD peptides to specifically target vascular endothelial growth factor (VEGF) over-expressing human umbilical vein endothelial cells (HUVECs) [17]. For a complete review on targeted delivery of siRNA for cancer therapies see [17].

Nanoparticle internalization

Many of the approaches used to enhance siRNA uptake and intracellular trafficking have been adopted from pDNA delivery. Naked siRNA with its relatively large molecular weight (~13 kDa) and polyanionic nature is not able to diffuse freely across the cell membrane. Attempts to penetrate the cell membrane directly without using the endosomal pathway include the direct conjugation of siRNAs to cell penetrating peptides (CPPs) [18, 19] and cholesterol [9, 18]. Although these approaches are hypothesized to avoid the endosomal pathway, their exact mechanism of entry was not studied. Nevertheless, both CPPs and cholesterol conjugation has been shown to enhance the efficiency of siRNA gene silencing in vivo [9, 18]. Most siRNA nanoparticles, however, enter the cell through endocytosis,

which poses a major limitation to siRNA efficiency since it must be released into the cytosol in order to activate the RNAi pathway. In general, siRNA nanoparticles (in vitro) enter more than 90% of the cells through endocytosis, however, the release of the nanoparticles and/or siRNA to the cytosol occurs less readily. Nanoparticle modification with ligands for receptor mediated endocytosis has shown to not only improve nanoparticle targeting but, consequently, internalization [12, 17].

Endosomal escape

Following internalization of a nanoparticle, an early endosome is formed, which is accompanied by acidification of the vesicular lumen that continues into the late endosomal and lysosomal compartments, reaching a final pH in the perinuclear lysosome of approximately 4.5. In addition, a redox potential exists between the extracellular and the endosomal intracellular space due to the presence of molecules like glutathione (GSH) and thioredoxin inside the endosome [20]. Thus, strategies to enhance endosomal escape have exploited either pH responsive or reduction sensitive polymers. pH responsive polymers include polymers that contain protonable amines that disrupt the endosomal membrane through the proton sponge effect [1, 21] or polymers that undergo hydrophilic-to-hydrophobic transitions and induce endosome membrane lysis [22]. Of the pH responsive polymers, poly(ethylene imine) (PEI) is the most widely used (for reviews see [23, 24]). Polymers that contain imidazoles have been recently reviewed [25]. Fusogenic peptides, natural hydrophilic-to-hydrophobic membrane destabilizing agents, have been covalently incorporated into siRNA/lipid and siRNA/cationic polymer complexes to enhance escape from the endosome [26, 27]. Fusogenic peptides, derived from influenza virus hemagglutinin protein, have been conjugated to a commercially available cationic lipid (Lipofectamine 2000) and showed up to a 3.5-fold enhancement of mRNA degradation compared to controls without the fusogenic peptide [26]. Amphiphatic polyanions based on dimethylaminoethyl methacrylate (DMAEMA), propylacrylic acid (PAA), and butyl methacrylate (BMA) [22] and amphiphatic polycations based on butyl and amino vinyl ethers [12] have been previously investigated because they possess endosomolytic behavior. The ratio of the different monomers determines the effectiveness of the endosomolytic response at a given pH. Both types of amphiphatic polymers were able to enhance the effectiveness of siRNA delivery through enhanced endosomal escape of the siRNA [12, 22]. Polymers that are reduction sensitive are typically generated through the use of a disulfide bond. These bonds are readily reduced to free thiols in the endosome. The use of reduction sensitive polymers to enhance endosomal escape and overall siRNA delivery has been recently reviewed [20].

Interestingly, while the current paradigm for the design of siRNA delivery vectors include strategies to induce endosomal escape, new research from the Langer group indicates that while 95% of internalized siRNA/lipid nanoparticles occurs through endocytosis, the remaining 5%, which enters through a different pathway, results in the majority of functional siRNA in the cytosol [28]. More specifically, they found that regardless of whether or not clathrin-, caveolin-, or lipid-raft-mediated endocytosis or macropinocytosis was inhibited, there was no significant change in target mRNA knockdown. However, these results still need time to be validated and the exact mechanism of entry identified before

long-held beliefs regarding the mechanism of siRNA/lipid nanoparticle internalization can be altered.

siRNA off target effects

The final and possibly most important step when using the RNAi pathway for therapeutic applications is the specific recognition and binding of siRNA in the cytosol to the appropriate mRNA for effective gene silencing. While it has been shown that a single base mismatch over the length of the siRNA is enough to block the response [3], other recent studies have indicated that it is possible for siRNA to target unintended mRNA through partial mismatch or sense strand interactions [29]. Off target effects may include, but are not limited to, inflammation including interferon response, cell toxicity, saturation of the endogenous mRNA pathway, and unintended effects on the target gene [29]. Several researchers have also shown that chemical modification of the siRNA guide strand can be used to increase specificity to target mRNA and reduce off-target effects [4, 30], including annealing of the guide strand to a passenger strand to reduce off-target effects mediated by passenger strand complementarity [30].

Approaches for siRNA delivery

Since the introduction of siRNA in 1998, researchers have identified the need for a delivery system to enhance the effect of exogenously delivered synthetic siRNA. Although there was some early success with the delivery of “naked” siRNA, the use of specific peptides, polymers, lipids, and other types of nanoparticles have shown significant progress in the development of siRNA as an effective therapeutic. These delivery vectors will be highlighted below and summarized in Table 3.

Naked siRNA

Despite the aforementioned issues concerning siRNA instability in serum, several early studies have had some success delivering naked siRNA [34, 35]. To increase the cellular permeability of naked siRNA manual hydrodynamic injection [34] and electrophoresis [35] have been used. Generally these methods are not ideal for in vivo applications and, in comparison, systemic delivery of siRNA nanoparticles, which have the ability to target and penetrate into desired cells and maintain siRNA integrity, is more favorable.

Direct conjugation to siRNA (less than 10nm in size)

The smallest siRNA nanoparticles result from the direct conjugation of small molecules, peptides or polymers to the sense strand of siRNA. Modifications of the sense strand do not appear to affect the ability of siRNA to degrade mRNA since it is the anti sense strand that recognizes the mRNA for degradation. Conjugation of siRNA to CPPs [18, 19] and PEG [31, 36] has increased gene transfer in vivo. The conjugation of cholesterol to the sense strand of siRNA improved slicing of multiple genes in mice, including endogenous apolipoprotein B gene expression in the liver and jejunum [9] and p38 mitogen-activated protein (MAP) kinase in the lungs [18]. Long chain fatty acids (> C_{18}) and bile-salt derivatives have also been conjugated to siRNA and shown in vivo gene silencing to hepatocytes [37]. Acid responsive polymers that contain PEG and a NAG targeting ligand

have been directly conjugated to siRNA making a “Dynamic PolyConjugate” and were shown to result in vivo gene silencing [12]. Upon entry into the endosome the acid responsive polymer releases the PEG and the targeting ligand, makes a hydrophilic to hydrophobic transition, and results in endosomal disruption.

Cationic polymers (100 to 300nm in size)

As mentioned the generation of larger nanoparticles is used to further prevent siRNA degradation, allow for longer circulation times and target specific tissues/cells. Cationic polymers such as poly(ethylene imine) (PEI) have been widely utilized for gene delivery and have been translated to siRNA delivery [38, 39]. These polymers form nanoparticles through the electrostatic interactions between the negatively charged phosphate groups in the siRNA backbone and the positive charges on the polymer. However, due to fundamental differences between pDNA and siRNA, including size and site of action, those polymeric vectors which have worked so well in the past with pDNA need to be modified to achieve similar efficiency when delivering siRNA. Chemically modified versions of PEI (e.g. PEI-PEG [40]) and other polymers, such as cyclodextrin-containing polycations, polylysine and natural polymers such as chitosan, have been made and shown enhanced efficiency for siRNA delivery [23, 24]. For a complete review on non-viral polymers used for siRNA delivery see [23, 24]. Peptides and proteins have also recently been used to complex siRNA to avoid the cytotoxic effects seen with the use of larger polymers [41]. Cationic peptides, such as CADY [42] and MPG-8 (a variant of MPG) [43], have been shown to efficiently complex siRNA. A novel CPP-modified protein was able to bind directly to siRNA, shield it from degradation, and deliver it to various cell lines in vitro [44].

Cationic lipids (100 to 300nm in size)

Lipid vectors have been shown to work more efficiently with siRNA, while the reason for this mechanistic difference is not clear. Some hypothesize the weaker interaction between lipids and siRNA which allows for faster de-complexation in the cytosol to contribute to this observation. Several lipid-based transfection agents are commercially available and being used in vivo to study the mechanism and effects of siRNA delivery in mammalian cells. These include, but are not limited to, jetSi-ENDO, Lipofectamine RNAiMAX, siPORT NeoFX, DharmaFECT, X-tremeGENE, and TriFECTin. DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane, was one of the first lipid formulations to be examined for in vivo siRNA delivery [23]. An alternative approach to the one-by-one synthesis and screening of cationic lipids for siRNA delivery which used a combinatorial library was recently described. A combinatorial library of 1,200 lipid-like materials, termed lipidoids, was developed for the delivery of siRNA and modified ODNs [45]. Lipidoids were synthesized upon conjugate addition of alkyl-acrylates or alkyl-acrylamides to primary or secondary amines. A number of materials were identified to substantially improve silencing over delivery of naked siRNA in vitro and in vivo in mice, rats, and nonhuman primates.

Neutral liposomes (<200nm in size)

To circumvent the potential toxicities associated with cationic polymers [41] and lipids [31], neutral liposomes which encapsulate siRNA are commonly being utilized. Unilammelar liposomes with hydrophilic cores and hydrophobic surfaces effectively shield encapsulated

siRNA from degradation by surrounding RNAses and facilitate internalization via membrane fusion or (receptor-mediated) endocytosis [16]. Recently Yagi, et. al sandwiched siRNA between a cationic core composed of DOTAP and an outer lipid bilayer of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(polyethylene glycol-2000) (PEG-DSPE) and egg phosphatidylcholine (egg-PC) and showed that it remained in circulation even 20hrs post-injection [46]. Stable nucleic acid lipid particles, SNALPs, represented a major advancement in lipid-based siRNA delivery after injection of clinically relevant doses of ApoB-siRNA produced knockdown in the liver of non-human primates [10].

Other nanoparticles

While cationic polymers, lipids, and liposomes have been shown to efficiently deliver siRNA, researchers are still looking to develop new types of nanoparticles to overcome the numerous limitations posed by the delivery of siRNA. Hyaluronic acid (HA) nanoparticles, also known as nanogels, with encapsulated siRNA were able to target HCT-116 cells in vitro which over-express HA-specific CD44 receptors [15]. Depending on the engineered nanogel characteristics, the siRNA release rate was also regulated. siRNA has also been incorporated in poly(D,L-lactic-co-glycolic) acid (PLGA) [33] and calcium carbonate [32] nanoparticles.

siRNA delivery from tissue engineering scaffolds

The use of siRNA to down regulate genes that in turn result in the expression of one or more tissue-inductive factors or the removal a factor that inhibits regeneration could be an ideal approach to guide the regeneration of tissue in vivo and in vitro. For example, the down regulation of PHD2, a prolyl-hydrozylase that tags HIF-1α for degradation during normoxia, has been shown to increase the amount of active HIF-1α, which in turn upregulates VEGF [47] and iNOS [47], two potent proangiogenic molecules that could aid the generation of stable leak free vessels [47]. Other examples of siRNA targets for use in tissue engineering include GNAS1 to induce bone formation, NgR to induce nerve regeneration and HOXB13 to aid in wound healing [47]. Although siRNA targets have been identified, the delivery of siRNA from tissue engineering matrices has not been extensively studied. Delivery of macromolecules from tissue engineering matrices has been proposed to enhance delivery in vivo by delaying clearance from the desired tissue, protecting the payload from degradation, and extending opportunities for internalization. Thus, siRNA delivery would benefit from controlled release from tissue engineering scaffolds. For example, siRNA delivery from alginate and collagen hydrogels exhibited sustained gene knockdown for 6 days [48]. The current techniques used to deliver DNA from tissue engineering scaffolds may be adapted to the delivery of siRNA [49].

In vivo therapeutic success

Several clinical studies using siRNA for therapeutic gene silencing are currently underway. One of the first siRNA therapies to enter clinical trials targeted VEGF for the treatment of wet neovascular age-related macular degeneration, now currently in phase III clinical trials [41]. Transferrin-tagged, cyclodextrin-based polymeric nanoparticles, named CALAA-01, which contain siRNA targeting the M2 subunit of ribonucleotide reductase for cancer treatment have recently entered phase I trials. This is the first targeted and systemic delivery

system for siRNA cancer therapy in clinical trials and has already reported to have shown efficient targeting and internalization [41]. For more information on early clinical studies using siRNA see [41]. For other therapeutic targets currently investigated see Table 4.

Conclusion

Within the past decade, the use of siRNA for RNAi has proven to be an effective nanomedicine for therapeutic gene silencing. Numerous delivery vectors which have either been adapted from traditional gene delivery or siRNA specific vectors have been developed and shown to have in vivo success. Although matrix based delivery has been investigated mostly for regenerative medicine applications, which aims to regenerate tissue by implanting biocompatible and biodegradable scaffolds at sites of injury, it is promising for the use of RNAi. siRNA delivery from tissue engineering matrices may also increase the number of cells exhibiting mRNA knockdown, while minimizing the quantity of vector used, an important factor when dealing with doss-dependant toxicity. Yet despite the limitations which still exist with the use of siRNA for RNAi, several clinical trials targeting specific tissues and diseases are currently taking place.

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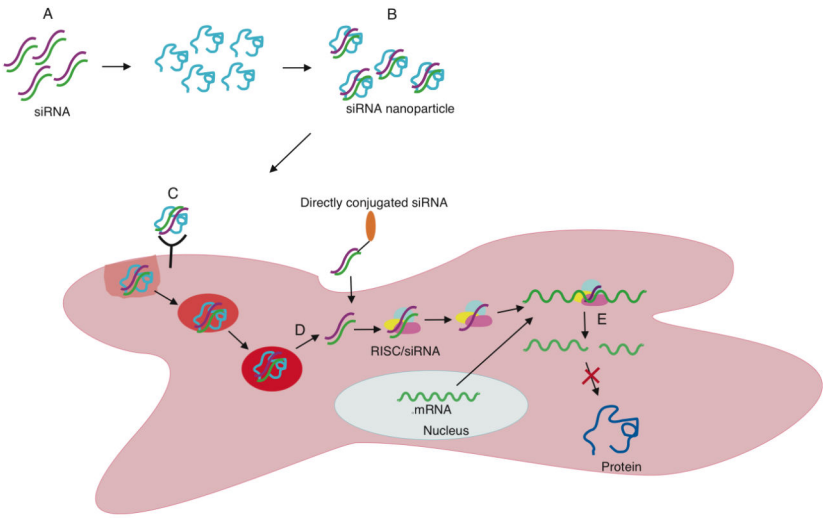

Figure 1.

Schematic of the limitations to siRNA delivery and the siRNA induced RNAi pathway. Limitations to siRNA delivery include: (A) siRNA stability, (B) siRNA nanoparticle stability, (C) siRNA or siRNA nanoparticle targeting and internalization, (D) siRNA endosomal escape, and (E) siRNA off-target effects.

Table 1

Methods for gene silencing

Gene Silencing Mechanism	Description	Stability in Serum	Notes	Ref
Oligonucleotides	18–25 bp, single-stranded; hybridizes to corresponding mRNA	Less resistant than siRNAs	Non-recyclable	[1, 6]
Ribozymes	Catalytically active RNA composed of 3 helices; catalyze cleavage of self or other RNA by trans-esterification or hydrolysis	10 seconds – few minutes	Stability may be too low for therapeutics	[1, 6]
RNAi				
siRNA Small interfering RNA	21–25 nt, double-stranded, endogenous or exogenous, natural defense against viruses and transposons, regulate mRNA degradation post-transcriptionally with perfectly matched sequences	Several minutes – 1 hour	100–1000 fold more efficient than ODNs	[1, 4]
shRNA Short hairpin RNA	Similar to ds-siRNA with a 4–9 nt loop at one end, processed by DICER to produce siRNA, transcribed by RNA polymerase III	More durable than siRNA	Can be introduced through pDNA	[5, 6]
miRNA Micro RNA	20–24 nt, endogenous, transcribed by RNA polymerase II, regulate mRNA degradation or translation inhibition post-transcriptionally with partial mis-matched sequences	-	Essential in animal development	[1, 5, 6]
piRNA Piwi-interacting RNA	24–30 nt, endogenous, bind to Piwi proteins, involved in transposon control, germline development, spermatogenesis	-	Highly abundant in testes	[7]

Table 2

Extracellular and intracellular limitations of RNAi using siRNA

Limitation	Possible Solution(s)	Ref
Extracellular		
Degradation in serum	•Chemical modification with PEG, etc.	[1, 4, 5, 11, 31]
	•Peptide/polymer/lipid complexation	[9, 18, 23, 24]
	•Nanoparticle encapsulation	[32, 33]
Targeting to specific cells	•Vector modification with targeting ligands	[12–14, 17]
Internalization	•Peptide/polymer/lipid complexation for charge neutralization	[23, 24]
	•Conjugation or complexation with CPPs	[18, 19]
	•Ligand modification for receptor mediated endocytosis	[12, 17]
Intracellular		
Endosomal escape	•Conjugation or complexation with fusogenic peptides	[26, 27]
	•Acid-responsive polymers/lipid complexation	[12, 22]
mRNA targeting	•Chemical modification of siRNA	[4, 30]

Table 3

Select examples of non-viral vectors used successfully to deliver siRNA in vivo

Name (Abbreviation)	Size (nm)*	In vivo Delivery	Notes	Ref
Direct Conjugation				
Tat(48–60)	< 10	Intratracheal	Peptide	[18]
Penetratin	< 10	Intratracheal	Peptide	[18]
Poly(ethylene glycol) (PEG)	< 10	Intravenous injection	Polymer	[12]
Ligand-PEG-poly(vinyl ether) (Dynamic Polyconjugates)	-	Intravenous injection	Polymer with targeting capability	[12]
Cholesterol	< 10	Intratracheal, Intravenous injection	Enhances stability and internalization	[9, 18]
High-density and low-density lipoproteins (HDL, LDL)	-	Intravenous injection	Long chain fatty and bile acids	[37]
Cationic Polymers				
Poly(ethylenimine) (PEI)	100–200	Intraperitoneal	Most widely used polymer	[39]
Poly(ethylenimine)-g-poly(ethylene glycol) (PEI-PEG)	200–500	Intravenous injection	PEG lowers cytotoxicity of PEI	[40]
Cyclodextrin-containing polycations	70–140	Intravenous injection	Other formulations exist	[14]
Chitosan	40–600	Intranasal	Natural polymer	[24]
MPG-8	70–170	Intratumoral, Intravenous injection	Peptide; variant of MPG	[43]
Cationic Lipids				
1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)	-	Intravenous injection	May also take the form of a liposome	[4]
Lipidoids	-	Intravenous injection	1,200 types in library	[45]
Liposomes and Nanoparticles				
Stable nucleic acid lipid particle (SNALP)	77–83	Intravenous injection	Composed of 4 different lipids	[10]
DOTAP/Egg-PC/PEG-DSPE (Wrapsome)	~100	Intravenous injection	Wraps siRNA between 2 layers	[46]
Phosphatidylcholine (PC), dipalmitoylphosphatidylethan olamine (DPPE), cholesterol liposome	~100	Intravenous injection	3:1:1 (PC:DPPE:Chol)	[16]
Poly(lactic-co-glycolic acid) (PLGA)	< 200	Topical	Polymer nanogel	[33]
Calcium carbonate	50–60	Intratumoral injection	New to siRNA delivery	[32]

* Size when vector is conjugated to or complexed with siRNA

Table 4

Select examples of therapeutic siRNA used successfully to silence target genes in vivo

Target (Disease)	Target Gene	siRNA Sequence (sense strand)	Ref(s)
Heart (Restenosis, Atherscleroticplaque rupture)	MMP2	5′-UCAUCGUCGUAGUUGGUUG-3′	[50]
Lung (Chronic obstructive pulmonary disease)	MAPK14	5′-GGGAGGUGCCCGAACGAUAUU-3′	[18]
Liver (High cholesterol)	ApoB	5′-GUCAUCACACUGAAUACCAA* U-3′	[9]
Brain (Epilepsy, Schizophrenia, Parkinson's)	GAD67	5′-GUAGAGACACCCUAAAGUAUU-3′	[51]
White blood cells (Inflammatory bowel disease, Colorectal dysplasia)	Cy-D1	5′-ACACCAAUCUCCUCAACGAUU-3′ 5′-GCAUGUUCGUGGCCUCUAAUU-3′ 5′-GCCGAGAAGUUGUGCAUCUUU-3′ 5′-GCACUUUCUUUCCAGAGUCUU-3′	[16]
Abdominal adhesion prevention	HIF-1α	5′-UAUUUGUUCACGUUAUCAGUU-3′ 5′-AAUACAUUGACCAUAUCGCUU-3′ 5′-CAACUCAGUAAUCCUUUCAUU-3′ 5′-UAAUUCACACACACAAUGCUU-3′	[38]
Tumor (xenograft)	Cy-B1	5′-GGCGAAGAUCAACAUGGCATT-3′	[43]
Tumor (allograft)	VEGF	5′-CGAUGAAGCCCUGGAGUGCTT-3′	[52]
Tumor (allograft)	KLF5	5′-AAGCUCACCUGAGGACUCATT-3′	[46]

*Represents phosphorothioate linkages