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Reviews

Non-viral vectors for the mediation of RNAi

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ABSTRACT

Though the delivery of siRNA into cells, tissues or organs remains to be a big obstacle for its applications, recently siRNA therapeutics has developed rapidly and already there are clinical trials ongoing or planned. Some non-viral vectors have attracted much more attention and shown the great potential for combating the delivery obstacle. As a novel class of lipid like materials lipidoids have the advantages of easy synthesis and large library of compounds. Cell penetrating peptides and chitosans have been used for the delivery of bioactive molecules for many years, but they are showing great promise for the delivery of siRNA. The hybrids of inorganic particles and the conjugates of siRNA have indicated the complex utilization different materials may provide another solution to the delivery problem. The most exciting thing is some clinical trials are undergoing, which provokes the hope of real curing method by using RNAi mediated by some non-viral vectors.

Contents

1. Introduction

RNA interference (RNAi), a naturally occurring process that mediates sequence specific inhibition of gene expression through the activation of RNA-induced silencing complex (RISC) by interaction with the small duplex RNA molecules termed siRNA [1], has been recognized "one of the most exciting discoveries in biology in the last couple of years" [2], since Fire et al. [3] have demonstrated that double-stranded RNA is much more effective at producing interference than either strand individually and that interference occurs at the posttranscriptional level, after successful par-1 suppression by injecting single stranded sense and antisense RNA [4]. The process of RNAi is related to a normal defense against viruses and the mobilisation of transposable genetic elements [5]. RNAi is triggered by small interfering RNA (siRNA), short RNA duplexes with a common length of 21–23 nt being generated from long dsRNAs of exogenous or endogenous origin [6–10], in

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this process long dsRNAs are recognized by a dsRNA-specific endonuclease (a cytoplasmic ribonuclease III (RNase III)-like protein) called Dicer, an enzyme which can cleave long dsRNA into siRNA of 21–23 nt in length.

RNAi technology is being evaluated as a potentially useful method to cure diseases including cancer, infection, respiratory disease, neuronal disease, and autoimmune disease [2,11]. RNAi shows many advantages over other therapeutic methods as very specific, without the toxic effects often observed during chemotherapy and the sequence-independent toxic effects of antisense therapy, and siRNA are more resistant to nuclease degradation than antisense oligonucleotides and, therefore, exhibiting longer therapeutic effects than antisense therapy [12].

However, the delivery of siRNA remains to be the biggest challenge, because it is imperative for siRNA to reach the cytoplasm of the targeted cells to become effective and induce silencing. As naked RNAs cannot penetrate cellular lipid membranes by themselves [13], siRNA must be enclosed in carriers such as viral vectors and non-viral vectors to be transported to the targeted cells *in vitro* or *in vivo*. The successful application of siRNA, is largely dependent on the development of a delivery vehicle which should be administered efficiently, safely, and repeatedly. Viral systems usually give high transfection efficiencies, safety concerns from potential mutation, recombination, oncogenic effect, and high cost, however, greatly limit their therapeutic applications. In contrast, non-viral vectors are believed to cause less safety problems due to their relative simplicity, though nonspecific cytotoxicity associated with cationic liposomes has been observed [14–16].

We could see many non-viral systems for successful delivery of siRNA both *in vitro* and *in vivo* including in nonhuman primates and humans [11,17–24] have been developed in recent years, though RNAi therapy has met great challenge during the past decade. Many reviews [25–29] have shown us the promising future of siRNA delivery by using lipid-based systems, chitosans, polymeric micelles, siRNA conjugates and peptide delivery systems. Herein we show the readers the recent development of siRNA delivery by using lipidoids, chitosans, cell penetrating peptides, inorganic particles and siRNA conjugates, and new progress in clinical trials. Based on these we could realize that RNAi is approaching the real need through the non-viral delivery method.

2. Lipidoids

Lipids have long been used for the delivery of genes and siRNAs, since the first cationic lipid (*N*-[1-(2,3,-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride) (DOTMA) was introduced by Felgner et al. in 1987. Many studies also show that cationic liposomes hold great promise as vehicles for effective human gene therapy. Many high quality reviews [30–32] with respect to cationic liposomes for gene therapy are available; we ever reviewed cationic compounds used for gene therapy [33]. In our article, many lipids have been described based on the chemical structure nature of these compounds; here we only introduce lipidoids to show the prosperous future of lipids for the delivery of siRNA.

Recently, a new approach to the synthesis (Fig. 1) lipid-like materials for siRNA delivery vectors using combinatorial methods was reported [34]. This one-step synthetic scheme enabled the straightforward parallel generation of large libraries of delivery materials. In this study, a library of over 1200 lipid-like materials, termed lipidoids, were generated through the conjugate addition of an amine to an α,β-unsaturated carbonyl and evaluated for siRNA delivery performance. The safety and efficacy of lipidoids were evaluated in three animal models: mice, rats and nonhuman primates. Therapeutic efficacy was observed *in vivo* in liver, lung and peritoneal macrophages. The studies suggest that these materials may have broad utility for both local and systemic delivery of RNA therapeutics.

Huang et al's results [35] suggest that lipidoid-formulated CLDN3 siRNA has the potential as a therapeutic for ovarian cancer, in which they have proved the efficacy of lipidoid-formulated CLDN3 siRNA in three different ovarian cancer models, by intratumoral injection to result in dramatic silencing of CLDN3, significant reduction in cell proliferation, reduction in tumor

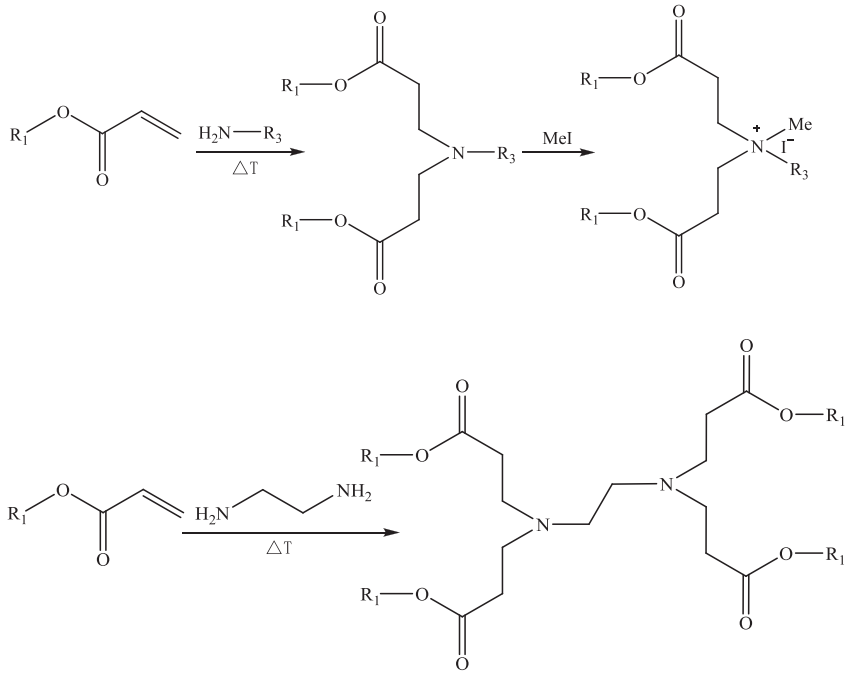

Fig. 1. Synthesis schematic of lipidoids by the conjugate addition of amine to α,β-unsaturated carbonyl compounds.

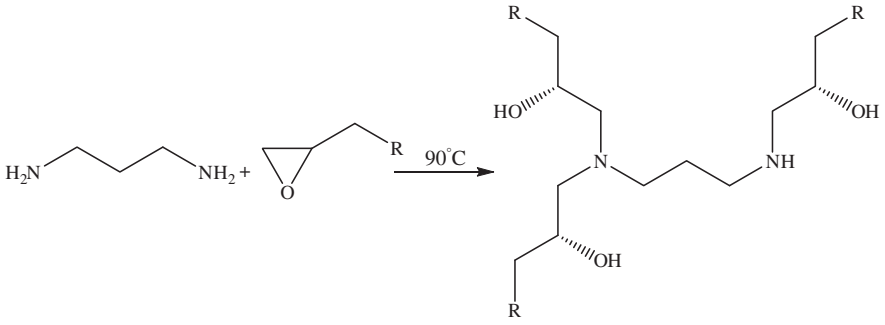

Fig. 2. Synthesis schematic of lipidoids by the conjugate addition of amine to epoxides.

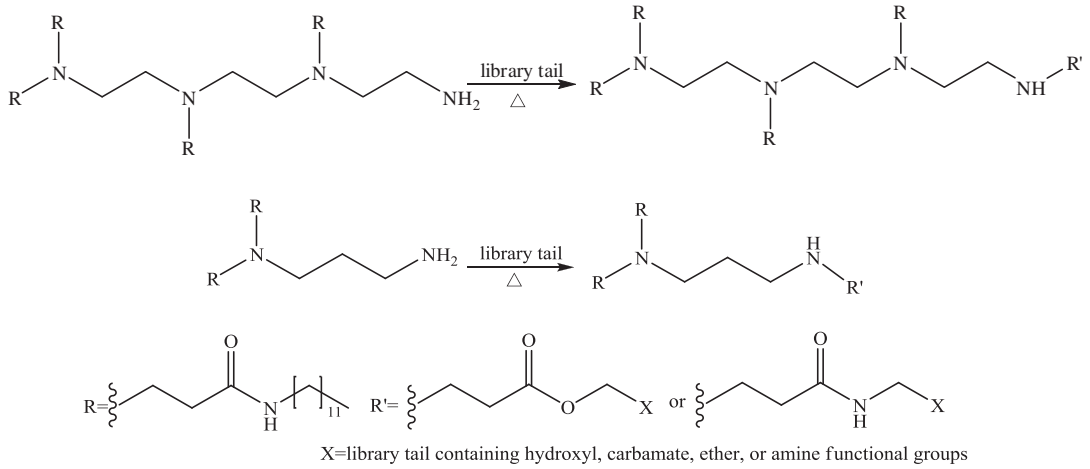

Fig. 3. Synthesis schematic of lipidoids by the conjugate addition of amine to lipid-like tails containing hydroxyl, carbamate, ether, or amine functional groups.

growth, and a significant increase in the number of apoptotic cells, and by intraperitoneal injection to result in a substantial reduction in tumor burden in MISIIR/Tag transgenic mice and mice bearing tumors derived from mouse ovarian surface epithelial cells.

Through the combinatorial synthesis (Fig. 2) and screening of a different class of materials, a formulation has been identified by Love et al. [36], which enables siRNA-directed liver gene silencing in mice at doses below 0.01 mg/kg and inhibits the expression of five hepatic genes simultaneously, after a single injection. The potential of this formulation was further validated in nonhuman primates, where high levels of knockdown of the clinically relevant gene transthyretin were observed at doses as low as 0.03 mg/kg.

Further, they synthesized materials of lipid-like tails and feature appendages containing hydroxyl, carbamate, ether, or amine functional groups as well as variations in alkyl chain length and branching (Fig. 3). The relationship between lipid chemical modification and delivery performance *in vitro* was studied using a luciferase reporter system in HeLa cells to show the impact of the functional group depending on the overall amine content and tail number of the delivery vectors [37].

3. Cell-penetrating peptides

Since Frankel and Pabo [38] found that the exogenous addition of full-length HIV-1 TAT protein to cells in culture resulted in cell membrane penetration and trans-activation of the HIV-1 promoter, many cell-penetrating peptides (CPPs) have been obtained, such as transportan [39], HSV-1 protein VP22 [40] and MPG [41], model amphipathic peptide (MAP) [42] and polyarginine [43] (Table 1). CPPs have been shown to be very promising to deliver siRNA, as they could significantly improve cellular uptake of various therapeutic molecules both in cultured cells and *in vivo* [44–45]. Some review articles [44,46–47] have summed up two main classes of CPPs studied: (1) the covalent linkage of the cargo to the CPP, thereby forming a conjugate which is achieved by either chemical crosslinking, cloning or expression of a protein fused to the CPP; (2) the formation of a non-covalent complex between

Table 1
Peptide sequences.

Peptides		Sequences
Transportan		GWTLNSAGYLLGKINLKALAALAKKIL-amide
HSV-1 VP22 peptide		DAATATRGRSAASRPTERPRAPARSASRPRRVD
MAP		KLALKLALKALKAALKLA-amide
MPG		GALFLGFLGAAGSTMGAWSQPKKKRKV-cysteamide
$MPG^{\Delta NLS}$		GALFLGFLGAAGSTMGAWSQPKSKRKV-cysteamide
Pα		GALFLGFLGAAGSTMGAWSQPKSKRKV-cysteamide
Pep-1		KETWWETWWTEWSQPKKKRKV-cysteamide
Pep-2		KETWFETWFTEWSQPKKKRKV-cysteamide
Pep-3		KETWFETWFTEWSQPKKKRKV-cysteamide
Polyarginine	R4	RRRR-Gly-cysteamide
	R6	RRRRRR-Gly-cysteamide
	R8	RRRRRRRR-Gly-cysteamide
	R10	RRRRRRRRRR-Gly-cysteamide
	R12	RRRRRRRRRRRR-Gly-cysteamide
	R16	RRRRRRRRRRRRRRRR-Gly-cysteamide
CADY		GLWRALWRLLRSLWRLLWRA-cysteamide

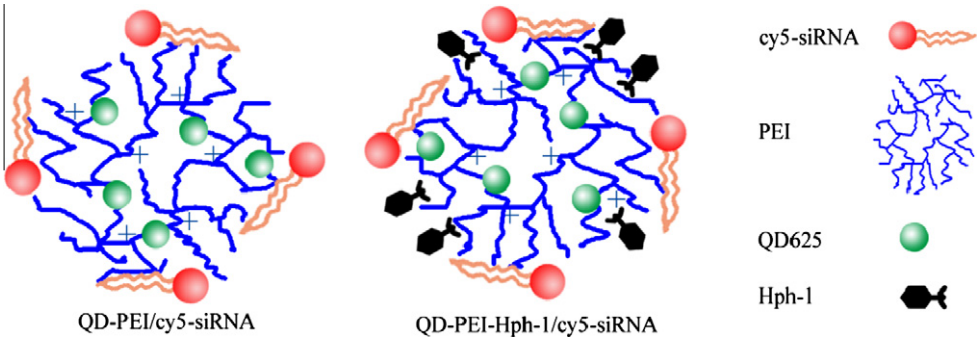

Fig. 4. Schematic illustration of QD-PEI and QD-PEI-Hph-1 conjugates.

the two partners. The first required chemical linkage with the cargos and the second involved the formation of stable, noncovalent complexes [48–51].

Meade and Dowdy [50] have stated that at the right molar and charge ratios, packaging of nucleic acids and CPPs resulted in insoluble complexes that contain an overall positive charge, aiding in cell membrane translocation. The benefits of this methodology come in both the low cost of reagents and the ease of preparation of CPP/siRNA complexes. MPG peptide with a peptide/siRNA complexing strategy was used by Simeoni et al. [52] to show successful siRNA introduction into cells. Later, Unnamalai et al. [53] have shown the successful induction of RNAi in tobacco cells by using a simple CPP/siRNA packaging scheme.

Recently, Crombez et al. [54] designed a secondary amphipathic peptide (CADY) of 20 residues combining aromatic tryptophan and cationic arginine residues, which could improve cellular uptake of siRNA into challenging cell lines, as CADY adopted a helical conformation within cell membranes, thereby exposing charged residues on one side, and Trp groups that favor cellular uptake on the other. They demonstrated that CADY was not toxic and the ability to support a sustained silencing response with low nanomolar concentrations of siRNA. They concluded that CADY entered cells through a mechanism which was independent of the major endosomal pathway. Later they combined molecular modeling, spectroscopy, and membrane interaction approaches to demonstrate that CADY formed stable complexes with siRNA and binds phospholipids tightly, mainly through electrostatic interaction. They proposed that CADY cellular membrane interaction was driven by its structural polymorphism which enabled stabilization of both electrostatic and hydrophobic contacts with surface membrane proteoglycan and phospholipids [55]. In a study, PEI conjugated QDs (quantum dots) were modified with a protein transduction domain (PTD) from human transcriptional factor, Hph-1(cell penetrating peptide). The two siRNA/QD-PEI complexes with and without Hph-1 have shown markedly different intracellular uptake behaviors and unpacking kinetics of cy5-siRNA (Fig. 4) [56].

Covalent linkage of CPPs to siRNA molecules offers the promising potential for CPP-mediated siRNA delivery. Disulfide bond is often shown to be optimal, it is easy for the carrier to release free siRNA duplexes upon exposure to the reducing cytoplasmic environment. Both penetratin (Antp) and transportan CPPs were attached to siRNA duplexes through disulfide bond by Muratovska and Eccles [57]. Their results indicated that CPP/siRNA complexes were more efficient than cationic liposome delivery with Lipofectamine to support the potential for these peptides to deliver siRNA into cells that are untransfectable with standard transfection reagents. Chiu et al. [58] also used a disulfide bond to introduce TAT CPP to siRNA. They found that TAT/siRNA conjugates showed maximum uptake at 300 nM comparable to the cy3 signal obtained from transfection of 300 nM cy3-labeled siRNA with Lipofectamine.

4. Chitosans

Chitosan, a naturally occurring cationic polysaccharide, has been shown to be biocompatible, non-inflammatory, nontoxic and biodegradable (Fig. 5) [59–61]. Chitosan has been widely used in drug delivery systems, especially for DNA-mediated gene therapy [62–64], because the protonated amine groups allow transport across cellular membranes [65] and subsequent endocytosis into cells. Since Mumper [66] pioneered to apply chitosan to gene delivery system in 1995, a variety of chitosans of high molecular weight and low molecular weight or their derivatives have been used to mediate gene delivery into various cell types. Numerous studies on DNA and siRNA delivery with chitosan as a carrier biomaterial have shown effective expression and silence reporter genes *in vitro* and *in vivo* [67–68].

Howard et al. [69] showed nanoparticles could be formed through electrostatic bridges between chitosan polymeric chains, and be used to mediate knockdown of the disease-associated BCR/ABL-1 protein. Western analysis showed ~90% reduced expression of BCR/ABL-1 leukemia fusion protein while BCR expression was unaffected in K562 (Ph^+) cells and effective *in vivo* RNA interference was achieved in bronchiole epithelial cells of transgenic EGFP mice after nasal administration of chitosan/siRNA formulations. The study showed the potential use of this chitosan-based system for mucosal RNA interference applications. Liu et al. [70] found that chitosan/siRNA nanoparticles formed using high molecular weight (M_w, 114 and 170 kDa) and degree of deacetylation (DD, 84%) chitosan formed at N:P 150 were the most stable and exhibited about 80% *in vitro* gene knockdown. Another type of chitosan nanoparticles were prepared by ionic cross-linking and ionic gelation using sodium tripolyphosphate (TPP) to indicate chitosan-TPP nanoparticles with entrapped siRNA were to be better vectors as siRNA delivery vehicles compared to chitosan-siRNA complexes possibly due to their high binding capacity and loading efficiency [71]. Mittnacht et al. [72] used chitosan/siRNA nanoparticles to facilitate effective cellular uptake of siRNA to immobilize these nanoparticles on neuronal microimplants to enable neurite outgrowth in the presence of inhibitory myelin. This is believed to be the first combinatorial approach of siRNA nanotherapeutics

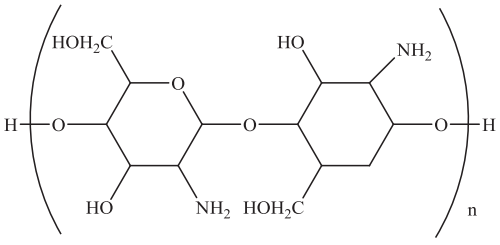

Fig. 5. Chemical structure of chitosan.

and biomaterial implants for the regeneration of the nervous system, from which RhoA nanoparticles resulted in a 65–75% RhoA mRNA reduction in PC12 cells compared to cells transfected with scr nanoparticles.

5. The hybrids based on inorganic particles

5.1. Gold nano-particles

Various nanosized inorganic particles have been utilized for the development of siRNA conjugates with both of therapeutic and diagnostic purposes. Investigation of siRNA conjugates with inorganic particles is of great interest to the development of more efficient methods for transfection of siRNA. Due to good biocompatibility, easy synthesis, monodispersity, and ready functionalization, gold nanoparticles have emerged as an attractive candidate for delivery of DNA and siRNA into cells [73–77]. Giljohann et al. [75] have developed the first polyvalent RNA–gold nanoparticle conjugates through the connection of ethylene glycol spacer and an alkylthiol group, which can be used to effectively regulate genes in the context of RNA interference. Lee et al. [76] developed a novel nanoparticulate delivery system based on gold nanoparticles modified with PEG, small interfering RNA conjugated to the nanoparticles via biodegradable disulfide linkages and poly(β-amino ester)s coating on the surface of the nanoparticles. The delivery system facilitated high levels of *in vitro* siRNA delivery. Elbakry [77] used monodisperse gold nanoparticles as a template for the manufacture of a siRNA layer-by-layer carrier which was assembled by the deposition of 11-mercaptoundecanoic acid (MUA) on the gold surface, the addition to PEI solution and siRNA and the completion of shell through PEI offered a unique opportunity to fabricate well-defined and homogenously distributed nanocarriers for siRNA delivery. Guo et al. [78] prepared charge-reversal functional gold nanoparticles (PEI/PAH-Cit/PEI/AuNPs) first by layer-by-layer technique to deliver small interfering RNA (siRNA) and plasmid DNA into cancer cells, suggesting the charge reversion under acidic environment facilitated the escape of gold nanoparticle/nucleic acid complexes from endosome/lysosome and release of functional nucleic acids into cytoplasm (Fig. 6).

A new concept [79] in using inorganic engineered nanoparticles in nucleic acid packaging and delivery applications was developed with low generation polypropylenimine (PPI) dendrimers by using Au NPs as a "labile catalytic" packaging agent. The Au NPs helped low generation dendrimers to package nucleic acids into discrete nanoparticles but were not included in the final DNA/siRNA complexes to eliminate the potential toxicity associated with Au NPs by selectively removing the Au NPs from the resulting nucleic acid complexes before their delivery to targeted cells. The efficiency of mRNA silencing by this novel approach was superior to generation 5 dendrimers.

5.2. Magnetic nanoparticles

Among all types of nanoparticles, magnetic nanoparticles, such as iron oxide, have been investigated for both drug delivery and magnetic resonance imaging [80]. These magnetic iron oxide nanoparticles with proper surface architecture and conjugated targeting ligands/proteins have attracted great attention for biomedical applications [81–83]. Liong et al. [84] synthesized multifunctional iron oxide mesoporous silica nanoparticles that were detectable by both MR imaging and optical methods. In a study [85], primary amine groups on the iron oxide nanoparticles were activated with a heterofunctional cross-linker, m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), and then conjugated to the thiol group of the antisense strand of siRNA. Near-infrared (NIRF) Cy5.5 dye and membrane translocation peptides were also coated onto magnetic nanoparticles. Tumor tissues were successfully visualized by magnetic resonance imaging (MRI) and NIRF image due to the accumulation of the resultant magnetic nanoparticle–siRNA conjugate at the tumor tissue after intravenous injection. The mRNA level of target genes (green fluorescence protein and human survivin) was suppressed to below 15% by systemic injection of the magnetic nanoparticle–siRNA conjugate, compared to saline-treated controls. Although siRNA was conjugated onto magnetic nanoparticles via a noncleavable bond (thioether) in this experiment, surprisingly significant RNAi activity was achieved *in vivo*.

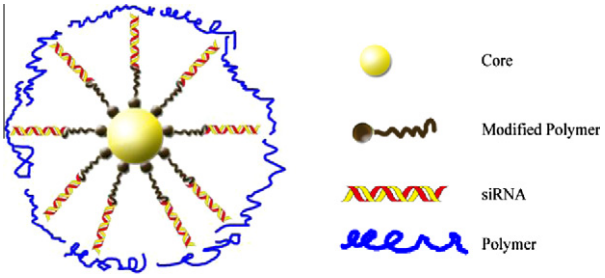

Fig. 6. Schematic illustration of the hybrids based on inorganic particles.

5.3. QDs and UCN

Among these inorganic particles semiconductor quantum dots (QDs) have been paying great attention. They have emerged as an alternative fluorescence resonance energy transfer (FRET) donor for proximal organic acceptor dyes [86]. In comparison with organic dyes, QDs have several unique intrinsic optical properties including size-tunable photoluminescent emission, broad absorption spectra, and large Stokes Shifts [87–90]. More recently, it was shown that QDs can be utilized to monitor the intracellular delivery and decomplexation of plasmid DNA (pDNA) [91].

Positively charged polyethylenimine (PEI) was covalently conjugated on the surface of QDs to complex with cyanine dye labeled vascular endothelial growth factor siRNA (cy5-VEGF siRNA), and FRET was achieved between cy5-VEGF siRNA and PEI conjugated QDs (QD625) to demonstrate that PEI conjugated QDs can be utilized as a useful siRNA carrier to analyze intracellular trafficking and unpacking pathway as well as to effectively silence a target gene (Fig. 4) [56].

Recently, upconversion FRET technique has emerged to eliminate some drawbacks of QDs including the strong autofluorescence from cells and biomolecules for limiting the sensitivity of FRET assay and the absorption of ultraviolet or visible excitation light by biological samples for attenuating the excitation of the donor, as the straightforward and sensitive upconversion FRET technique can gain real-time information on intracellular fate of siRNA and provide a bright outlook for *in vitro* and even *in vivo* detection of biological molecules. In a study, the UCN/siRNA-BOBO3 complex was prepared where BOBO-3-stained siRNA was attached to the surface of amino-group-modified silica/NaYF4:Yb,Er UCN. The energy was transferred from the UCN donor to the BOBO-3 acceptor under excitation of a near-infrared (NIR) laser. Intracellular FRET analysis showed that siRNA was gradually released into cells for the duration of 24 h [92].

6. Chemical conjugates of siRNA

Chemical modification of siRNA by using small drug molecules, aptamers, lipids, peptides, proteins, or polymers could increase its intracellular delivery and avoid its degradation by RNA nucleases

[93]. Therefore, the conjugates could significantly enhance biological half-life with a concomitant increase of delivery efficiency to the target tissue while maintaining sufficient gene silencing activity [26].

6.1. Disulfide bond linked

The reducible disulfide bond is commonly used for the linkage between siRNA and the modified parts in the conjugate structures. In a study [94], the double-stranded GFP-siRNA with a phosphothioethanol (PE) portion was reversibly modified via the reducible disulfide bond and incorporated the resulting siRNA-S-S-PE conjugate in nanosized PEG-PE micelles which showed good stability against nucleases and release easily from these nanoparticles. The mixed GFP-siRNA-S-S-PE/PEG-PE micelles could down-regulate the GFP production 50-fold more effectively than free siRNA in GFP-C166 endothelial cells, and showed no toxicity (Fig. 7).

The covalent conjugation of siRNA to certain cell-penetrating peptides (CPPs), such as Penetratin [95], HIV-1 Tat [96] and Transportan [97], to enhance cell delivery has shown to be effective [98–99]. In this modification, disulfide-linked conjugates of siRNA with a range of cationic and other CPPs were synthesized by Turner et al. [100]. Though steric block inhibition of gene expression for conjugates of CPPs with a 12-mer oligonucleotide mixmer of 2′-O-methyl and locked nucleic acids units was not obtained, reduction in expression of P38α MAP kinase mRNA in HeLa cells using μM concentrations of Penetratin or Tat peptides conjugated to the 3′-end of the sense strand of siRNA was found. Park et al. [101–107] conjugated polyethylene glycol (PEG) to siRNA via a reducible disulfide linkage, and they further complexed PEG–siRNA conjugate with cationic polymers or peptides as core condensing agents to form colloidal nanoparticles for the delivery vehicle for local and systemic treatments for tumors in an animal model.

6.2. Lipophilic modification

The lipophilic conjugates of siRNA have been believed to induce intracellular RNAi without the significant loss of gene silencing activity. Lorenz et al. [108] synthesized two series of lipophilic siRNAs conjugated with derivatives of cholesterol, lithocholic acid or lauric acid which were covalently linked to the 5′-ends of the RNAs using phosphoramidite chemistry. Their results showed that siRNA with a modified sense strand downregulated β-galactosidase expression to a higher extent than siRNA with a modified antisense strand or two modified strands and modified siRNA did not reduce gene expression under identical conditions.

A siRNA with chemically modified backbone conjugated to a lipophilic cholesterol moiety at the 3′ end of the sense strand led to suppression of apolipoprotein B mRNA by approximately 60%

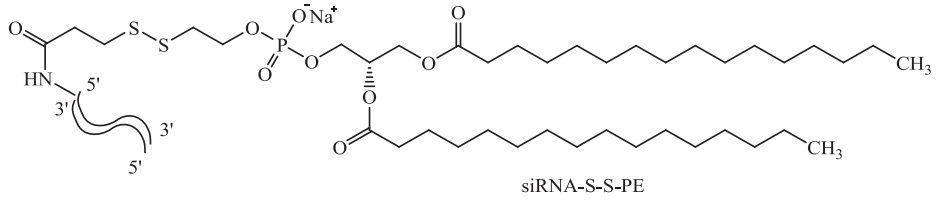

Fig. 7. Chemical structure of siRNA-S-S-PE.

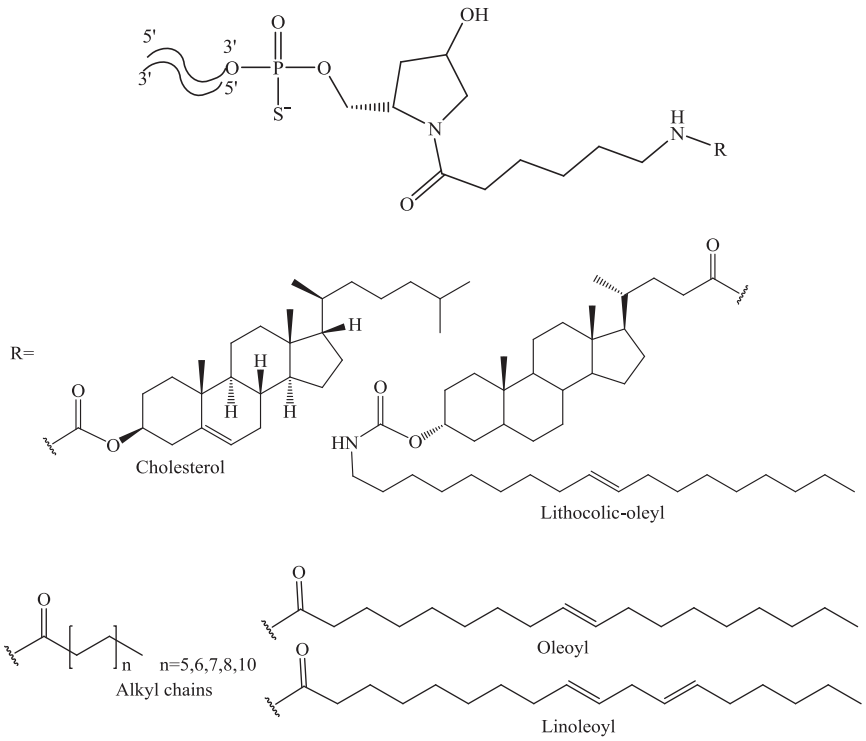

Fig. 8. Structure of lipophile–siRNA conjugates.

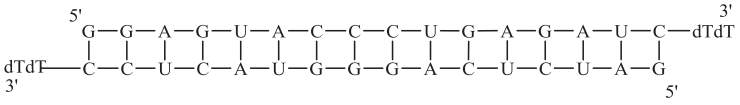

Fig. 9. The sequence of the VEGF-targeting siRNA.

in the liver after iv injection. Soutschek et al. [109] reported that no immune stimulation or off-target effect occurred in mice at a high dose of 50 mg/kg. Recent studies [110] indicated that the cholesterol-conjugated siRNA was delivered into the hepatocytes as a complex of lipoproteins (Fig. 8). Targeted delivery of siRNA via the linkage to ligand peptide, aptamer and antibody to the desired cells and tissues has been considered as an attractive way to bring siRNA drugs to clinical settings. For this part readers are recommended for referring a comprehensive review by Jeong et al. [26].

7. Clinical trials mediated by non-viral vectors

Synthetic siRNAs for human clinical trials were firstly used in direct intraocular siRNA injections for patients with blinding choroidal neovascularization (CNV). Subsequently, other clinical trials have initiated and early clinical data are beginning to appear [111–113]. Since Acuity Pharmaceuticals performed the first siRNA protocol granted investigational new drug (IND) status and tested in a human clinical trial, in which the vascular endothelial growth factor (VEGF)-targeted siRNA Bevasiranib was used for the treatment of wet age-related macular degeneration (Fig. 9) [111], we have experienced great progress in RNAi therapy mediated by non-viral vectors for clinical trials.

Koldehoff et al. [114] reported on the *in vivo* application of targeted non-virally delivered synthetic *bcrabl* siRNA in a female patient with recurrent Philadelphia chromosome-positive chronic myeloid leukaemia (CML) resistant to imatinib (Y253F mutation) and chemotherapy after allogeneic haematopoietic stem cell transplantation. A remarkable inhibition of the overexpressed *bcr-abl* oncogene resulting in increased apoptosis of CML cells and no any clinically adverse events were found. The findings implied that the clinical application of synthetic siRNA was feasible, safe and has real potential for genetic-based therapies using synthetic non-viral carriers.

In 2008, a phase I trial using the experimental therapeutic CALAA-01 was initiated, and it is believed that the first targeted delivery of siRNA was accomplished in a human. The first patient was treated in May of 2008 [115]. Later, patients with solid cancers refractory to standard-of-care therapies were administered doses of targeted nanoparticles on days 1, 3, 8 and 10 of a 21-day cycle by a 30-min intravenous infusion. They used a TF (transferrin protein) targeted, cyclodextrin and AD-PEG (adamantine–polyethyleneglycol) nanoparticle delivery system to administer systemically siRNA to patients with solid cancers to provide evidence of inducing an RNAi mechanism of action in a human from the delivered siRNA. They also found the presence of an mRNA fragment that demonstrates that siRNA-mediated mRNA cleavage occurs specifically at the site predicted for an RNAi mechanism from a patient who received the highest dose of the nanoparticles [116].

8. Conclusions

As an extremely powerful research tool RNAi has many advantages over other therapeutic methods and holds significant potential for a wide variety of gene silencing applications. Non-viral vectors have attracted more and more attention in comparison to viral vectors, as they have the advantages over non-viral vectors such as ease of synthesis, low immune response against the vector and unrestricted gene materials size in addition to potential benefits in terms of safety. Lipidoids have been synthesized by using combinatorial methods, this scheme enabled the straightforward parallel generation of large libraries of delivery material through the conjugate addition of an amine to an α,β-unsaturated carbonyl. Lipidoids have been characterized by the very low dose (0.03 mg/kg) used *in vivo*, thus making the safety problem agreeable to human beings. Cell penetrating peptides and chitosans have been used for the delivery of bioactives for many years, but they are showing great promise for the delivery of siRNA. CPPs have been shown to be very promising to deliver siRNA, as they could significantly improve cellular uptake of various therapeutic molecules both in cultured cells and *in vivo*. A variety of chitosans of high molecular weight and low molecular weight or their derivatives have been used to mediate gene delivery into various cell types. Numerous studies on DNA and siRNA delivery with chitosan as a carrier biomaterial have shown effective expression and silence reporter genes *in vitro* and *in vivo*. Various nanosized inorganic particles have been utilized for the development of siRNA conjugates dual therapeutic and diagnostic purposes. Investigation of siRNA conjugates with inorganic particles is of great interest to the development of more efficient methods for transfection of siRNA. The conjugates of siRNA attached to other materials may provide another solution to the delivery problem, because the conjugates could significantly enhance biological half-life with a concomitant increase of delivery efficiency to the target tissue while maintaining sufficient gene silencing activity. The combination of CPPs and siRNA could show much higher efficiency than cationic liposomes to support the fact of CPPs enhanced siRNA delivery. Some clinical trials are improving our determination on the hope of real curing method by using RNAi mediated by some non-viral vectors.

Acknowledgments

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References

[1] J.C. Cheng, T.B. Moore, K.M. Sakamoto, Mol. Genet. Metab. 80 (2003) 121–128.
[2] S. Jana, C. Chakraborty, S. Nandi, J.K. Deb, Appl. Microbiol. Biotechnol. 65 (2004) 649–657.
[3] A. Fire, S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, C.C. Mello, Nature 391 (1998) 806–811.
[4] S. Guo, K.J. Kemphues, Cell 81 (1995) 611–620.
[5] M. Tijsterman, R.F. Ketting, R.H. Plasterk, Annu. Rev. Genet. 36 (2002) 489–519.
[6] P.D. Zamore, T. Tuschl, P.A. Sharp, D.P. Bartel, Cell 101 (2000) 25–33.
[7] E. Bernstein, A.A. Caudy, S.M. Hammond, G.J. Hannon, Nature 409 (2001) 363–366.
[8] S.M. Elbashir, J. Harborth, W. Lendeckel, A. Yalcin, K. Weber, T. Tuschl, Nature 411 (2001) 494–498.
[9] S.M. Elbashir, W. Lendeckel, T. Tuschl, Genes Dev. 15 (2001) 188–200.
[10] S.M. Elbashir, J. Martinez, A. Patkaniowska, W. Lendeckel, T. Tuschl, EMBO J. 20 (2001) 6877–6888.
[11] P.K. Leung, P.A. Whittaker, Pharmacol. Ther. 107 (2005) 222–239.
[12] D.M. Dykxhoorn, C.D. Novina, P.A. Sharp, Nat. Rev. Mol. Cell Biol. 4 (2003) 457–467.
[13] C.X. Li, A. Parker, E. Menocal, S. Xiang, L. Borodyansky, J.H. Fruehauf, Cell Cycle 5 (2006) 2103–2109.

[14] H. Farhood, R. Bottega, R.M. Epand, L. Huang, Biochim. Biophys. Acta 1111 (1992) 239–246.
[15] K. Romoren, B.J. Thu, N.C. Bols, O. Evensen, Biochim. Biophys. Acta 1663 (2004) 127–134.
[16] C.W. Scales, F. Huang, N. Li, Y.A. Vasilieva, J. Ray, A.J. Convertine, C.L. McCormick, Macromolecules 39 (2006) 6871–6881.
[17] F.Y. Xie, M.C. Woodle, P.Y. Lu, Drug Discovery Today 11 (2006) 67–73.
[18] S.E. Martin, N.J. Caplen, Annu. Rev. Genomics Hum. Genet. 8 (2007) 81–108.
[19] G. Liu, F. Wong-Staal, Q.X. Li, Histol. Histopathol. 22 (2007) 211–217.
[20] D.H. Kim, J.J. Rossi, Nat. Rev. Genet. 8 (2007) 173–184.
[21] A. de Fougerolles, H.P. Vornlocher, J. Maraganore, J. Lieberman, Nat. Rev. Drug Discovery 6 (2007) 443–453.
[22] D. Bumcrot, M. Manoharan, V. Koteliansky, D.W. Sah, Nat. Chem. Biol. 2 (2006) 711–719.
[23] A. Aigner, J. Biomed. Biotechnol. 2006 (2006) 1–15.
[24] L. Aagaard, J.J. Rossi, Adv. Drug Delivery Rev. 59 (2007) 75–86.
[25] K. Gao, L. Huang, Mol. Pharm. 6 (2008) 651–658.
[26] J.H. Jeong, H. Mok, Y.K. Oh, T.G. Park, Bioconjugate Chem. 20 (2009) 5–14.
[27] N. Nishiyama, K. Kataoka, Pharmacol. Ther. 112 (2006) 630–648.
[28] Y.C. Tseng, S. Mozumdar, L. Huang, Adv. Drug Delivery Rev. 61 (2009) 721–731.
[29] V. Biricova, A. Laznickova, Bioorg. Chem. 37 (2009) 185–192.
[30] M.C. Woodle, P. Scaria, Curr. Opin. Colloid Interface Sci. 6 (2001) 78–84.
[31] J. Zabner, Adv. Drug Delivery Rev. 27 (1997) 17–28.
[32] R.I. Zhdanov, O.V. Podobed, V.V. Vlassov, Bioelectrochemistry 58 (2002) 53–64.
[33] S.B. Zhang, Y.M. Xu, B. Wang, W.H. Qiao, D.L. Liu, Z.S. Li, J. Controlled Release 100 (2004) 165–180.
[34] A. Akinc, A. Zumbuehl, M. Goldberg, E.S. Leshchiner, V. Busini, N. Hossain, S.A. Bacallado, D.N. Nguyen, J. Fuller, R. Alvarez, A. Borodovsky, T. Borland, R. Constien, A. de Fougerolles, J.R. Dorkin, K.N. Jayaprakash, M. Jayaraman, M. John, V. Koteliansky, M. Manoharan, L. Nechev, J. Qin, T. Racie, D. Raitcheva, K.G. Rajeev, D.W.Y. Sah, J. Soutschek, I. Toudjarska, H.P. Vornlocher, T.S. Zimmermann, R. Langer, D.G. Anderson, Nat. Biotechnol. 26 (2008) 561–569.
[35] Y.H. Huang, Y.H. Bao, W.D. Peng, M. Goldberg, K. Love, D.A. Bumcrot, G. Cole, R. Langer, D.G. Anderson, J. Sawicki, PNAS 106 (2009) 3426–3430.
[36] K.T. Love, K.P. Mahon, C.G. Levins, K.A. Whitehead, W. Querbes, J.R. Dorkin, J. Qin, W. Cantley, L.L. Qin, T. Racie, M.F. Kamenetsky, K.N. Yip, R. Alvarez, D.W.Y. Sah, A. de Fougerolles, K. Fitzgerald, V. Koteliansky, A. Akinc, R. Langer, D.G. Anderson, PNAS 107 (2010) 1864–1869.
[37] K.P. Mahon, K.T. Love, K.A. Whitehead, J. Qin, A. Akinc, E. Leshchiner, I. Leshchiner, R. Langer, D.G. Anderson, Bioconjugate Chem. 21 (2010) 1448–1454.
[38] A.D. Frankel, C.O. Pabo, Cell 55 (1988) 1189–1193.
[39] M. Pooga, C. Kut, M. Kihlmark, M. Hallbrink, S. Fernaeus, R. Raid, T. Land, E. Hallberg, T. Bartfai, U. Langel, FASEB J. 15 (2001) 1451–1453.
[40] G. Elliot, P. O'Hare, Cell 88 (1997) 223–233.
[41] M.C. Morris, P. Vidal, L. Chaloin, F. Heitz, G. Divita, Nucleic Acids Res. 25 (1997) 2730–2736.
[42] J. Oehlke, A. Scheller, B. Wiesner, E. Krause, M. Beyermann, E. Klauschenz, M. Melzig, M. Bienert, Biochim. Biophys. Acta 1414 (1998) 127–139.
[43] S. Futaki, T. Suzuki, W. Ohashi, T. Yagami, S. Tanaka, K. Ueda, Y. Suguira, J. Biol. Chem. 276 (2001) 5836–5840.
[44] S. El-Andaloussi, T. Holm, U. Langel, Curr. Pharm. Des. 11 (2005) 3597–3611.
[45] L. Crombez, A. Charnet, M.C. Morris, G. Aldrian-Herrada, F. Heitz, G. Divita, Biochem. Soc. Trans. 35 (2007) 44–46.
[46] M.C. Morris, S. Deshayes, F. Heitz, G. Divita, Biol. Cell 100 (2008) 201–217.
[47] S. Deshayes, M. Morris, F. Heitz, G. Divita, Adv. Drug Delivery Rev. 260 (2008) 537–547.
[48] C.L. Murriel, S.F. Dowdy, Expert Opin. Drug Delivery 3 (2006) 739–746.
[49] E. Gros, S. Deshayes, M.C. Morris, G. Aldrian-Herrada, J. Depollier, F. Heitz, G. Divita, Biochim. Biophys. Acta 1758 (2006) 384–393.
[50] B.R. Meade, S.F. Dowdy, Adv. Drug Delivery Rev. 59 (2007) 134–140.
[51] L. Crombez, M.C. Morris, S. Deshayes, F. Heitz, G. Divita, Curr. Pharm. Des. 14 (2008) 3656–3665.
[52] F. Simeoni, M.C. Morris, F. Heitz, G. Divita, Nucleic Acids Res. 31 (2003) 2717–2724.
[53] N. Unnamalai, B.G. Kang, W.S. Lee, FEBS Lett. 566 (2004) 307–310.
[54] L. Crombez, G. Aldrian-Herrada, K. Konate, Q.N. Nguyen, G.K. Mc Master, R. Brasseur, F. Heitz, G. Divita, Mol. Ther. 17 (2009) 95–103.
[55] K. Konate, L. Crombez, S. Deshayes, M. Decaffmeyer, A. Thomas, R. Brasseur, G. Aldrian, F. Heitz, G. Divita, Biochemistry 49 (2010) 3393–3402.
[56] H. Lee, I.K. Kim, T.G. Park, Bioconjugate Chem. 21 (2010) 289–295.
[57] A. Muratovska, M.R. Eccles, FEBS Lett. 558 (2004) 63–68.
[58] Y.L. Chiu, A. Ali, C. Chu, H. Cao, T.M. Rana, Chem. Biol. 11 (2004) 1165–1175.
[59] S.C.W. Richardson, H.V.J. Kolbe, R. Duncan, Int. J. Pharm. 178 (1999) 231–243.
[60] K. Corsi, F. Chellat, L. Yahia, J.C. Fernandes, Biomaterials 24 (2003) 1255–1264.
[61] T. Chandy, C.P. Sharma, Biomater. Artif. Cell Artif. Organ. 18 (1990) 1–24.
[62] G. Borchard, Adv. Drug Delivery Rev. 52 (2001) 145–150.
[63] W.G. Liu, K.D. Yao, J. Controlled Release 83 (2002) 1–11.
[64] S. Mansouri, P. Lavigne, K. Corsi, M. Benderdour, E. Beaumont, J.C. Fernandes, Eur. J. Pharm. Biopharm. 57 (2004) 1–8.
[65] J.C. Verhoef, H.E. Junginger, M. Thanou, Adv. Drug Delivery Rev. 50 (2001) 91–101.
[66] R.J. Mumper, J. Wang, J.M. Claspell, A.P. Rolland, Proc. Int. Symp. Control. Rel. Bioact. Mater. 22 (1995) 178–179.
[67] T. Kiang, J. Wen, H.W. Lim, K.W. Leong, Biomaterials 25 (2004) 5293–5301.
[68] W.G. Liu, S.J. Sun, Z.Q. Cao, X. Zhang, K.D. Yao, W.W. Lu, K.D. Luk, Biomaterials 26 (2005) 2705–2711.
[69] [69] A.K. Howard, U.L. Rahbek, X.Liu, C.K. Damgaard, S.Z. Glud, M.Ø. Andersen, M.B. Hovgaard, A. Schmitz, J.R. Nyengaard, F. Besenbacher, J. Kjems, Mol. Ther. 14 (2006) 476–484.
[70] X. Liu, K.A. Howard, M. Dong, M.Ø. Andersen, U.L. Rahbek, M.G. Johnsen, O.C. Hansen, F. Besenbacher, J. Kjems, Biomaterials 28 (2007) 1280–1288.
[71] H. Katas, H.O. Alpar, J. Controlled Release 115 (2006) 216–225.
[72] U. Mittnacht, H. Hartmann, S. Hein, H. Oliveira, M. Dong, A.P. Pêgo, J. Kjems, K.A. Howard, B. Schlosshauer, Nano Lett. 10 (2010) 3933–3939.
[73] T. Niidome, K. Nakashima, H. Takahashi, Y. Niidome, Chem. Commun. (2004) 1978–1979.
[74] P.S. Ghosh, C.K. Kim, G. Han, N.S. Forbes, V.M. Rotello, ACS Nano. 2 (2008) 2213–2218.
[75] D.A. Giljohann, D.S. Seferos, A.E. Prigodich, P.C. Patel, C.A. Mirkin, J. Am. Chem. Soc. 131 (2009) 2072–2073.
[76] J.S. Lee, J.J. Green, K.T. Love, J. Sunshine, R. Langer, D.G. Anderson, Nano Lett. 9 (2009) 2402–2406.
[77] A. Elbakry, A. Zaky, R. Liebkl, R. Rachel, A. Goepferich, M. Breunig, Nano Lett. 9 (2009) 2059–2064.
[78] S. Guo, Y. Huang, Q. Jiang, Y. Sun, L. Deng, Z. Liang, Q. Du, J. Xing, Y. Zhao, P.C. Wang, A. Dong, X.J. Liang, ACS Nano. 4 (2010) 5505–5511.
[79] A.M. Chen, O. Taratula, D. Wei, H.I. Yen, T. Thomas, T.J. Thomas, T. Minko, H. He, ACS Nano. 4 (2010) 3679–3688.
[80] A.K. Gupta, M. Gupta, Biomaterials 26 (2005) 3995–4021.
[81] M. Mahmoudi, A.S. Milani, P. Stroeve, Int. J. Biomed. Nanosci. Nanotechnol. 1 (2010) 164–201.
[82] M. Mahmoudi, M.A. Shokrgozar, A. Simchi, M. Imani, A.S. Milani, P. Stroeve, H. Vali, U.O. Häfeli, P. Sasanpour, S. Bonakdar, J. Phys. Chem. C 113 (2009) 2322–2331.
[83] M. Mahmoudi, H. Hosseinkhani, M. Hosseinkhani, S. Boutry, A. Simchi, W.S. Journeay, K. Subramani, S. Laurent, Chem. Rev. 111 (2011) 253–280.
[84] M. Liong, M. Kovochich, T. Xia, S.G. Ruehm, A.E. Nel, F. Tamanoi, J.I. Zink, ACS Nano. 2 (2008) 889–896.
[85] Z. Medarova, W. Pham, C. Farrar, V. Petkova, A. Moore, Nat. Med. 13 (2007) 372–377.
[86] M. Howarth, K. Takao, Y. Hayashi, A.Y. Ting, PNAS 102 (2005) 7583–7588.
[87] W.C.W. Chan, S.M. Nie, Science 281 (1998) 2016–2018.
[88] I.L. Medintz, A.R. Clapp, H. Mattoussi, E.R. Goldman, B. Fisher, J.M. Mauro, Nat. Mater. 2 (2003) 630–638.
[89] C.J. Murphy, Anal. Chem. 74 (2002) 520A–526A.
[90] M. Bruchez, M. Moronne, P. Gin, S. Weiss, A.P. Alivisatos, Science 281 (1998) 2013–2016.
[91] H.H. Chen, Y.P. Ho, X. Jiang, H. Mao, T. Wang, K.W. Leong, Mol. Ther. 16 (2008) 324–332.
[92] S. Jiang, Y. Zhang, Langmuir 26 (2010) 6689–6694.
[93] S.D. Copley, E. Smith, H.J. Morowitz, Bioorg. Chem. 35 (2007) 430–443.
[94] T. Musacchio, O. Vaze, G. D'Souza, V.P. Torchilin, Bioconjugate Chem. 21 (2010) 1530–1536.
[95] A. Prochiantz, Curr. Opin. Neurobiol. 6 (1996) 629–634.
[96] E. Vivès, P. Brodin, B. Lebleu, J. Biol. Chem. 272 (1997) 16010–16017.
[97] M. Pooga, U. Soomets, M. Hällbrink, A. Valkna, K. Saar, K. Rezaei, U. Kahl, J.-X. Hao, X.-J. Xu, Z. Wiesenfeld-Hallin, T. Hökfelt, T. Bartfai, Ü. Langel, Nat. Biotechnol. 16 (1998) 857–861.
[98] D. Derossi, A.H. Joliot, G. Chassaing, A. Prochiantz, J. Biol. Chem. 269 (1994) 10444–10450.
[99] M.A. Lindsay, Curr. Opin. Pharmacol. 2 (2002) 587–594.
[100] J.J. Turner, S. Jones, M.M. Fabani, G. Ivanova, A.A. Arzumanov, M.J. Gait, Blood Cells Mol. Dis. 38 (2007) 1–7.
[101] S.H. Kim, J.H. Jeong, S.H. Lee, S.W. Kim, T.G. Park, J. Controlled Release 116 (2006) 123–129.
[102] J.H. Jeong, S.H. Kim, S.W. Kim, T.G. Park, Bioconjugate Chem. 16 (2005) 1034–1037.
[103] J.H. Jeong, S.W. Kim, T.G. Park, J. Controlled Release 93 (2003) 183–191.
[104] J.H. Jeong, S.W. Kim, T.G. Park, Bioconjugate Chem. 14 (2003) 473–479.
[105] M. Oishi, F. Nagatsugi, S. Sasaki, Y. Nagasaki, K. Kataoka, Chem. Bio. Chem. 6 (2005) 718–725.
[106] S.H. Lee, S.H. Kim, T.G. Park, Biochem. Biophys. Res. Commun. 357 (2007) 511–516.
[107] S.H. Kim, J.H. Jeong, S.H. Lee, S.H. Kim, T.G. Park, J. Controlled Release 129 (2008) 107–116.
[108] C. Lorenz, P. Hadwiger, M. John, H.-P. Vornlocher, C. Unverzagt, Bioorg. Med. Chem. Lett. 14 (2004) 4975–4977.
[109] J. Soutschek, A. Akinc, B. Bramlage, K. Charisse, R. Constien, M. Donoghue, S. Elbashir, A. Geick, P. Hadwiger, J. Harborth, M. John, V. Kesavan, G. Lavine, R.K. Pandey, T. Racie, K.G. Rajeev, I. Rohl, I. Toudjarska, G. Wang, S. Wuschko, D. Bumcrot, V. Koteliansky, S. Limmer, M. Manoharan, H.-P. Vornlocher, Nature 432 (2004) 173–178.
[110] C. Wolfrum, S. Shi, K.N. Jayaprakash, M. Jayaraman, G. Wang, R.K. Pandey, K.G. Rajeev, T. Nakayama, K. Charrise, E.M. Ndungo, T. Zimmermann, V. Koteliansky, M. Manoharan, M. Stoffel, Nat. Biotechnol. 25 (2007) 1149–1157.

[111] D. Castanotto, J.J. Rossi, Nature 457 (2009) 426–433.
[112] J. DeVincenzo, J.E. Cehelsky, R. Alvarez, S. Elbashir, J. Harborth, I. Toudjarska, L. Nechev, V. Murugaiah, A. Van Vliet, A.K. Vaishnaw, R. Meyers, Antiviral Res. 77 (2008) 225–231.
[113] S.A. Leachman, R.P. Hickerson, M.E. Schwartz, E.E. Bullough, S.L. Hutcherson, K.M. Boucher, C.D. Hansen, M.J. Eliason, G.S. Srivatsa, D.J. Kornbrust, F.J.D. Smith, W.I. McLean, L.M. Milstone, R.L. Kaspar, Mol. Ther. 18 (2010) 442–446.
[114] M. Koldehoff, N.K. Steckel, D.W. Beelen, A.H. Elmaagacli, Clin. Exp. Med. 7 (2007) 47–55.
[115] M.E. Davis, Mol. Pharm. 6 (2009) 659–668.
[116] M.E. Davis, J.E. Zuckerman, C.H.J. Choi, D. Seligson, A. Tolcher, C.A. Alabi, Y. Yen, J.D. Heidel, A. Ribas, Nature 464 (2010) 1067–1071.