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Progress and perspective of inorganic nanoparticles based siRNA delivery system

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Abstract

Introduction—Small interfering RNA (siRNA) is an effective method for regulating the expression of proteins, even “undruggable” ones that are nearly impossible to target through traditional small molecule therapeutics. Delivery to the cell and then to the cytosol is the primary requirement for realization of therapeutic potential of siRNA.

Areas covered—We summarize recent advances in the design of inorganic nanoparticle with surface functionality and physicochemical properties engineered for siRNA delivery. Specifically, we discuss the main approaches developed so far to load siRNA into/onto NPs, and NP surface chemistry engineered for enhanced intracellular siRNA delivery, endosomal escape, and targeted delivery of siRNA to disease cells and tissues.

Expert Opinion—Several challenges remain in developing inorganic NPs for efficient and effective siRNA delivery. Getting the material to the chosen site is important, however the greatest hurdle may well be delivery into the cytosol, either through efficient endosomal escape or by direct cytosolic siRNA delivery. Effective delivery at the organismic and cellular level coupled with biocompatible vehicles with low immunogenic response will facilitate the clinical translation of RNAi for the treatment of genetic diseases.

Keywords

Cancer therapy; Inorganic nanoparticle; RNAi; siRNA delivery; Surface chemistry

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Declaration of interest

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1. Introduction

RNA interference (RNAi) is a specific endogenous gene silencing pathway in which small RNA mediate the degradation of targeted mRNA (Figure 1) [1–3]. RNAi has been extensively utilized in the past decade [3,4] as a powerful tool for selectively controlling protein expression, even proteins that are normally considered to be nearly impossible to target using traditional small molecule therapeutics [5]. Previous research has successfully utilized RNAi as a tool for examining downstream effects from the knockdown of particular genes [6–8]. Therapeutic delivery of siRNA has also been shown to be effective in treating diseases like cancer [9–10]. However, there are many hurdles to overcome before realizing the therapeutic potential of RNAi. In particular, siRNA is an unstable negatively charged biomacromolecule and cannot effectively cross cell membranes, thus requiring a delivery vehicle [4, 11–12]. Traditionally, siRNA was introduced into cells *via* physical methods such as microinjection and electroporation [13]. However, these methods are not suitable for most *in vivo* uses. Virus-based delivery strategies are effective for delivery of siRNA [14–15], however they can induce insertional mutagenesis and have undesired immune response [16]. In recent years as RNAi research has shifted from fundamental studies towards applications for disease treatment, new methods of introducing siRNA are required.

A large number of non-viral vehicles have been developed for siRNA delivery, including cationic polymers [17], liposomes [18], virus-like particles [19], cell penetrating peptides [20], and inorganic nanoparticles [21]. Despite numerous efforts, very few efficient and effective approaches for siRNA delivery have emerged. The challenge is multifaceted: first the vehicle must effectively cross the cell membrane while avoiding or escaping endosomal entrapment [22–23]. The vector requires stability outside the cell, in particular from degradation by nucleases [4], but then effectively release the payload inside the cell.

Inorganic nanoparticles provide useful platforms for the engineering of RNA-based delivery vehicles for *in vitro* and *in vivo* applications [12, 23–29]. Inorganic NPs have high surface area to volume ratio, allowing for efficient loading of siRNA *via* direct conjugation or non-covalent encapsulation. A key advantage of these NPs is that their surface chemistry is easily modified, providing a means of overcoming the challenges of *in vitro* and *in vivo* siRNA delivery [21]. In addition, the unique physical and optical properties of inorganic NPs can be used to track siRNA delivery to cells or tissues [30–31]. There have been a wide range of inorganic NPs used for delivery (Table 1), and their advantages and disadvantages compared to organic NPs-based siRNA delivery system was summarized in Table 2 [6, 9, 20, 21, 24]; this review will focus on recent advances that we feel provide useful leads for therapeutic development.

2.1. Gold Nanoparticles

AuNPs have physical and chemical properties that make them perhaps the most studied inorganic NPs for siRNA delivery [27, 29–32]. First, the core of AuNP is essentially inert, non-toxic, and biocompatible, thus enabling a stable and safe siRNA delivery platform. Second, the well-developed surface chemistry of AuNPs allows the conjugation of siRNA *via* either covalent or non-covalent chemistry. Finally, the ability to “tune” AuNP properties

through surface modification provides useful properties for controlling NP delivery and release.

2.1.1. Covalent conjugation of AuNP/siRNA—A common approach for siRNA delivery involves the covalent conjugation of siRNA with various cell-penetrating or targeting ligands, such as lipids [18], peptides [20], and small molecule receptors [9]. These conjugates have shown effectiveness for siRNA delivery, with GalNAc-conjugated siRNA exhibiting promising results in silencing targeted gene in clinical trials [12]. However, these siRNA conjugates usually lack serum stability and have a short circulation time which prohibits a long term gene silencing effect. NP-based siRNA delivery approaches have shown superior stability against enzymatic degradation, and additionally prevent the premature release of siRNA into the physiological environment that can occur using other siRNA conjugates.

The most direct method to use AuNPs for siRNA delivery is to conjugate siRNA onto AuNP surface *via* thiol-gold covalent chemistry. Nagasaki *et al.* first used siRNA–AuNP conjugates for siRNA delivery in 2006 [33]. They modified 15 nm AuNPs with thiol–PEG₅₀₀₀–PAMA₇₅₀₀ polymer, followed by self-assembling thiolated siRNA. The resulting AuNP/siRNA conjugates led to a 65% gene silence efficiency with human hepatoma HuH-7 cells. Mirkin *et al.* has extended this concept to create spherical nucleic acid (SNA) nanoparticles (Figure 2a). SNAs are remarkably stable in physiological environments and resist nuclease degradation. They first conjugated 13 nm AuNPs with thiol-siRNA and oligoethylglycol thiol [34]. The use of a shorter PEG higher than 99% [35]. In subsequent studies, SNAs against Bcl2Like12 (Bcl2L12) oncogene were effective in knocking down endogenous Bcl2L12 mRNA and protein levels of glioma cells [36]. The systemic administration of SNAs reduced Bcl2L12 expression in intracerebral glioblastoma multiforme, increased intratumoral apoptosis, and reduced tumor burden and progression in xenografted mice. More recently, the same group demonstrated that SNAs dispersed in Aquaphor can penetrate the epidermal barrier of both intact mouse and human skin, enter keratinocytes, and efficiently silence target genes resulting in enhanced rates of wound healing [37].

Covalent siRNA/AuNP conjugates can be further coated with cationic polymer or cell penetrating peptides to enhance siRNA delivery efficiency. For example, Reich *et al.* first conjugated thiolate siRNA onto a gold nanoshell surface, followed by coating the NP surface with a streptavidin layer used to attach cell penetrating peptides through biotin-streptavidin ligation [38]. Upon irradiation with biocompatible near infrared (NIR) light, the siRNA was released from the gold surface and escaped from the endosome. The siRNA construct was efficiently internalized by a broad range of human embryonic stem cells (hESC), knocking down of Oct4 induced the differentiation of hESC to all three germ layers. Anderson *et al.* conjugated thiol-siRNA onto amine-coated AuNP using a dual-functional crosslinker, succinimidyl 3-(2-Pyridyldithio)Propionate (SPDP) [39]. The siRNA-AuNP conjugates were post-coated with biodegradable polymer, poly (β -amino ester), to improve siRNA gene silencing efficiency. The treatment of luciferase-expressing HeLa cells with 90 nM siRNA silenced gene expression in an efficiency higher than 90% in serum-containing medium.

The ease of functionalization of AuNPs makes them excellent platforms for targeted delivery vehicles. For example, the simultaneous conjugation of thiol-siRNA and Arg-Gly-Asp (RGD) onto AuNP efficiently delivered siRNA to the tumor site in a lung cancer orthotopic murine model [40]. Knocking down c-myc oncogene using this targeting siRNA/AuNP conjugate resulted in tumor growth suppression and prolonged survival of lung tumor bearing mice.

The simplicity and versatility of the AuNP scaffold allows the creation of complex machinery. Kim *et al.* reported an *i*-motif-driven gold nanoparticle-based nanomachine for programmed siRNA delivery [41]. They immobilized a pH-responsive nucleic acid consisted of three functional segments, i.e., an *i*-motif DNA, which has an acid-responsive switchable secondary structure, an overhanging linker DNA, and a therapeutic siRNA, on the nanomachine. After entering cells, the acidic endosome environment triggers the formation of an interstrand tetraplex of *i*-motif to induce cluster formation of AuNP, which facilitates endosomal escape and releases siRNA into cytosol. The delivery of siRNA against polo-like kinase 1 (PLK1) significantly reduced the viability of NIH3T3 cells, suggesting the therapeutic potential of this strategy.

2.1.2. Non-covalent encapsulation of siRNA/AuNP—Non-covalent delivery strategies provide a versatile modular strategy for the delivery of free siRNA. The negative charge of the siRNA strand facilitates assembly with positively charged NPs. For example, Rotello *et al.* have reported dendronized AuNPs for siRNA delivery (Figure 2b) [42]. AuNPs (2 nm core) were functionalized with dendronized ligands based on biodegradable glutamic cores with cationic triethylenetetramine (TETA) termini. The second-generation of dendronized AuNPs (G2-AuNPs) encapsulated siRNA with the highest efficiency. G2-AuNP delivered β -galactosidase targeting siRNA (β -gal-siRNA) into SVR-bag4 cells, with knockdown efficiency up to 50%, as measured by enzyme activity assay.

Cationic polymers, such as PEI and polylysine (PLL), have a long history as gene delivery vehicles. The direct functionalization of AuNP with these polymers enables encapsulation of siRNA and delivery. For example, Park *et al.* reported the controlled synthesis of PEI-coated AuNP using catechol-conjugated PEI for siRNA delivery [43]. Also, PEI has been used as both reductant and stabilizer to synthesize AuNPs which have been used to silence GFP and oncogenic PLK-1 gene *via* siRNA delivery for cancer therapy [44].

Layer-by-layer (LBL) coating of a gold core with cationic polymers and siRNA is another method for creating polymer-coated AuNPs for siRNA delivery. Negatively charged AuNPs were complexed with bPEI (25 kDa) and then sequentially deposited siRNA and bPEI to form AuNP/bPEI/siRNA/bPEI complexes, which showed an increased hydrodynamic diameter of 22 ~ 25 nm. On average, around 780 siRNA molecules were complexed with each AuNP, which was a significant enhancement compared to covalent siRNA-AuNP conjugates. The gene silencing efficiency of AuNP/bPEI/siRNA/bPEI complexes was verified by delivering siRNA against EGFP to CHO-K1 cells [45]. Hahn reported LBL assembled cysteamine (CM) modified AuNP (AuCM)/siRNA/PEI/hyaluronic acid (HA) for intracellular delivery of siRNA [46]. The introduction of HA into the NPs enabled the targeted delivery of siRNA to B16F1 cells which has HA receptors. The authors

demonstrated the capability of (AuCM)/siRNA/PEI/HA for *in vivo* siRNA delivery by knocking down apolipoprotein B expression in mouse liver.

The use of biodegradable biopolymers provides a means of reducing the toxicity of the carrier. For example, Tung *et al.* incorporated protease-degradable poly-lysine (PLL) and siRNA on the surface of AuNPs, and reported siRNA/PLL multilayer-coated AuNPs for siRNA delivery [47]. The PLL multilayer can be degraded by lysosomal cathepsin B enzyme to release siRNA gradually, inducing a prolonged gene-silencing effect. They later demonstrated that AuNPs with two layers of siRNA and three layers of PLL provided 20 days of > 60% luciferase knockdown in a MDA-MB-231-Luc xenograft [48].

More recently, “charge-reversal” polyelectrolytes have been applied to siRNA delivery. The “charge-reversal” polymer change net charge, from positive at neutral pH to negative under acidic environment efficiently controls siRNA release inside cells. For example, Liang *et al.* have used pH-responsive *cis*-aconitic anhydride-functionalized poly(allylamine) (PAH-Cit), and developed a “charge-reversal” LBL AuNP/siRNA delivery method (Figure 2c) [49]. AuNPs were initially modified with negatively charged 11-mercaptopundecanoic acid (MUA), upon which bPEI was deposited forming a cationic AuNP-MUA/bPEI core for LBL assembly. PAH-Cit and bPEI were then sequentially deposited onto the AuNP-MUA/bPEI core to encapsulate siRNA. The assembled PEI/PAHCit/PEI/MUA-AuNP/siRNA shows improved endosome escape efficiency and a higher gene knockdown efficiency than commercial lipid reagents and bPEI. Alternatively, charge-reversal chitosan (CS) can assemble similar LBL AuNP to deliver siRNA that silences the multidrug resist gene MDR1 [50], showing a potential pathway to overcome the drug resistance problem of traditional chemotherapy.

2.1.3. Strategies to facilitate endosome escape and direct cytosolic siRNA delivery—A key barrier to efficient siRNA delivery and effective RNAi is the delivery of siRNA cargo to the cytosol, where siRNA is loaded into the RISC to degrade mRNA. Nanomaterials usually enter cells via an endocytic pathway, resulting in endosome entrapment. It has been reported that even with relatively efficient vectors only a small fraction (1–2%) of internalized siRNA can escape from the endocytic pathway [51]. Designing nanocarriers that facilitate endosome escape of siRNA, or directly deliver siRNA into cytosol is therefore highly desired for effective RNAi [23, 52].

One commonly used method to facilitate endosomal escape of siRNA is to design highly positively charged polyamine carriers, which enhance endosome escape capability through the “proton sponge effect” [27]. These polyamine carriers, such as PEI and its derivatives, sequester protons when they enter the acidic lysosome compartment of cells due to the large buffering capacity of secondary or tertiary amines presented on the carriers. The continuous protonation of polyamine carriers subsequently pump chloride ions into the endosome, causing a water influx that leads to endosomal swelling and lysis, releasing siRNA into the cytoplasm [22, 23]. However, a recent study revealed that the widely used PEI played a limited role on enhancing endosome escape even under strong acidic lysosomal environment [53]. Introducing membrane-disruptive or fusogenic peptides such as GALA and KALA [54, 55], onto AuNP surface improves the endosomal escape of siRNA to certain extent, with

improved gene knockdown efficiency. Alternatively, hollow AuNPs that can be heated with NIR light irradiation have facilitated escape of siRNA from the endosome. For example, Li *et al.* demonstrated that conjugates of siRNA targeting NF- κ B p65 and hollow AuNP allowed for controllable cytoplasmic siRNA delivery upon NIR light irradiation [56].

A different strategy is to avoid endosomal uptake altogether. Rotello *et al.* recently developed a platform that provides direct cytosolic delivery of siRNA using nanoparticle-stabilized nanocapsules (NPSC) (Figure 3) [57]. In this approach, siRNA is complexed with cationic arginine-functionalized gold nanoparticles *via* electrostatic interactions, with the resulting ensemble self-assembling onto the surface of fatty acid nanodroplets to form a NPSC/siRNA nanocomplex. The complex rapidly delivers siRNA into cytosol in a cholesterol-dependent manner *via* membrane fusion. Live cell images and video show fluorescently-labeled siRNA was evenly distributed within the whole cell, with no punctate fluorescence indicative of endosomal uptake. This method delivered siRNA and silenced destabilized green fluorescent protein (deGFP) of HEK293 cells up to 90%. Moreover, the delivery of siRNA targeting PLK1 efficiently silenced oncogenic PLK1 expression in cancer cells showing the potential of this system for cancer therapy.

2.2. Magnetic nanoparticles

Magnetic nanoparticles (MNPs) provide useful properties for siRNA delivery. First, external magnetic fields can be used to manipulate the MNPs for spatially controlled siRNA delivery. Second, MNPs can be exploited with magnetic resonance imaging (MRI) to track siRNA delivery. Both of these properties were employed by Cheon *et al.*, who generated multifunctional MNPs (MnMEIO) by conjugating siRNA onto a MNPs surface. They first coated MNPs with serum albumin (BSA), followed by conjugation of fluorescent and thiolated siRNA using SPDP as a crosslinker (Figure 4a) [58]. In addition, an RGD peptide was introduced to target $\alpha_v\beta_3$ integrin overexpressing metastatic tumor cells. The targeted delivery of siRNA into $\alpha_v\beta_3$ integrin overexpressed MDA-MB-435 cells was verified by T2*-weighted MR imaging. MnMEIO/siGFP/RGD nanoparticle efficiently knocked down GFP expression in MDA-MB-435 cells, while no GFP knockdown was observed for $\alpha_v\beta_3$ integrin negative A549 cells under the same conditions. Similarly, Medarova *et al.* designed “all-in-one” MNPs for siRNA delivery and tumor imaging [59]. NIR fluorophore Cy5.5 and a peptide targeting tumor-specific antigen uMUC-1, were simultaneously conjugated to MNPs, enabling the visualization of siRNA delivery to tumor sites using MRI and NIR optical imaging.

Similar to AuNPs, surface modification of MNPs using cationic polymer and lipids allows electrostatic encapsulation of siRNA. Chen *et al.* coated magnetic iron oxide nanoclusters with cationic alkylated PEI, and used these nanoparticles for siRNA delivery *in vitro* and *in vivo* using an fLuc-4T1 xenograft model to demonstrate luciferase knockdown [60]. Magnetic nanocrystal clusters (PMNCs) crosslinked with PEI provide an alternative magnetic platform for siRNA delivery *in vitro* [61]. Bhatia *et al.* developed dendrimer-conjugated magnetofluorescent nanoworms, called “dendriforms” for siRNA delivery *in vivo* [62]. These siRNA-carrying dendriforms were internalized by cells with high endosomal escape and EGFR knockdown of 70–80%. Anderson *et al.* reported a simple method to load

and deliver siRNA by coating iron oxide nanoparticles with lipid-like molecules, called lipidoid [63]. The combinatorial synthesis of lipidoids allows the generation of a library of lipid-coated MNPs and for screening of the most effective siRNA delivery vehicles. One of the top lipid candidates for siRNA delivery, C14–200 coated MNPs, delivered siRNA and silenced luciferase expression of modified HeLa cells up to 80% at a siRNA concentration as low as 1.5 nM.

Magnetically-guided delivery provides a tool for spatial control of therapeutics. Namiki and co-workers assembled oleic acid-coated magnetic nanocrystals with cationic lipid formulations to generate LipoMag particles for siRNA delivery [64]. Intravenous injection of LipoMag, combined with the application of an external magnetic field, resulted in 50% reduction of tumor growth in gastric tumor bearing mice.

2.3. Mesoporous silica nanoparticles (MSNs)

Mesoporous silica nanoparticles (MSNs) have been used to deliver a variety of drugs, including small molecules, genes, and proteins [65]. The large surface area of MSNs provides space and binding sites to accommodate siRNA *via* non-covalent encapsulation or direct covalent conjugation. For example thiolated siRNA was efficiently conjugated to MSN surfaces for delivery. Also, coating MSNs with cationic polymers (e.g., polyethyleneimine, polyamidoamine, and polylysine) has been demonstrated to be efficient in encapsulating siRNA via electrostatic interactions [66, 67, 68]. Nel *et al.* coated MSNs with PEI and studied the effect of polymer size on cellular uptake of MSNs. They found that 10 KD PEI-coated MSNPs were capable of delivering siRNA and knocking down GFP expression of modified HEPA-1 liver cancer cells without cytotoxicity. [66]. In another approach, Gu *et al.* encapsulated siRNA into the mesoporous pores of MSNs, followed by surface coating with PEI and a fusogenic peptide (KALA) to enhance the endosomal escape of MSNs [55]. The resulting MSN/siRNA complex protected siRNA from degradation, had low toxicity, and was efficiently internalized by cells. The efficacy of this system was demonstrated by delivery of siRNA targeting vascular endothelial growth factor (VEGF) into tumor-bearing mice, effectively suppressing tumor growth. To reduce the cytotoxicity associated with the use of direct b-PEI-coated MSNs for delivery, Lellouche *et al.* used cerium (III) cations (Ce^{3+}) to crosslink bPEI to poly-amine modified MSNs. It was concluded that Ce^{3+} -nitrogen (from the amines of MSNs and PEI) play critical roles in attaching the bPEI shell onto MSNs surfaces for siRNA delivery [69].

The coating of PEI onto MSNs surface provides additional primary amine groups to conjugate targeting ligands for cancer cell targeted siRNA delivery. For example, anti-HER2 monoclonal antibody (trastuzumab)-conjugated PEI/MSNs selectively deliver siRNA into HER2-positive cells [70]. The delivery of siRNA selectively induced apoptotic death of HER2-positive BT474 cells, but not HER2-negative MCF-7 breast cancer cells. The administration of targeted PEI/MSNs/siRNA (against HER2 oncogene) into orthotopic HCC1954 tumor bearing mice effectively suppressed tumor growth.

The pores of MSNs allow the accommodation of small molecules, while the MSNs surface can be engineered with cationic polymers to encapsulate siRNA via electrostatic binding, providing a vehicle for co-delivery of a drug and siRNA for synergistic therapy. Chen *et al.*

designed a MSN-based co-delivery method by loading doxorubicin into large capacity MSNs [71]. The MSN exterior was then modified with G2 amine-terminated polyamidoamine (PAMAM) dendrimers for siRNA encapsulation. The co-delivery of doxorubicin and siRNA targeting Bcl-2 gene to MDR cancer cells significantly restored the sensitivity of the cells toward doxorubicin treatment, compared to that without siRNA delivery. Coating of MSNs with folic acid (FA) enabled the co-delivery of doxorubicin and Bcl-2 siRNA into FA-positive HeLa cells with enhanced therapeutic efficiency [72]. Finally, Zink *et al.* demonstrated that the co-delivery of doxorubicin and siRNA against P-glycoprotein (Pgp) drug exporter silenced P-gp expression in a MCF-7/MDR xenograft model in nude mice, resulting in a synergistic inhibition of tumor growth *in vivo* (Figure 4b) [73].

2.4. Calcium phosphate nanoparticles (CaP)

Calcium phosphate (CaP) is a natural inorganic material found in human bone and teeth with promise for drug delivery due to its biocompatibility and biodegradation ability. CaP can encapsulate negatively charged gene cargos through the chelation of calcium ions and the phosphate of genes, forming CaP nanoparticles that are internalized by cells [74]. However, CaP-based siRNA delivery currently has challenges arising from the low endosome escape efficiency of CaP and uncontrollable growth of CaP/siRNA NPs under physiological environment.

Kataoka *et al.* self-assembled a pH-responsive block polymer, poly(ethylene glycol)-block-poly(methacrylic acid) (PMA), into CaP/siRNA NPs to enhance endosomal escape of CaP vehicles. PMA becomes more hydrophobic under an acidic environment (pH = 4–6), disrupting the endosome membrane and releasing siRNA [75]. The authors report a high gene knockdown efficiency using PMA/CaP/siRNA NPs to silence luciferase in modified human embryonic kidney 293 cells. Similarly, Jeong *et al.* the natural anionic polymer hyaluronic acid (HA), conjugated with dioleoylphosphatidic acid (DOPA) to control the size and stability of CaP/siRNA NPs (Figure 5a) [76]. The CaP/siRNA/DOPA-HA NPs maintain the integrity and stability of encapsulated siRNA, and showed improved intratumoral accumulation of siRNA and a high level of target gene silencing in solid tumors after systemic administration. Huang *et al.* used an alternate strategy for stabilizing CaP nanoparticle, using an asymmetric lipid bilayer for tumor targeted siRNA delivery [77]. PEGylation and functionalization of the above CaP NPs with targeting anisamide ligands enabled the delivery of siRNA to human H460 lung cancer xenografts with improved *in vivo* gene silencing efficiency.

3. Conclusions and prospects

Inorganic nanoparticles provide a diverse set of platforms for siRNA delivery. The broad range of chemical approaches for engineering NP surfaces provides the ability to further optimize siRNA delivery *in vitro* and *in vivo*. In addition, the unique physical and optical properties of inorganic NPs have been used to track siRNA delivery and bio-distribution, and for the development of theranostic agents. The therapeutic potential of inorganic NPs for siRNA delivery has been demonstrated in a broad range of disease models, providing

promise for future therapeutic applications. However, there are remaining hurdles that are preventing the progression of this delivery mechanism to clinical trials, evidenced by the fact that there are no currently active clinical trials using inorganic nanoparticles for RNAi therapeutics. Engineering and manufacturing siRNA-loaded inorganic nanoparticles with homogenous size, composition, and surface charge in a simple, fast, and inexpensive manner require concerted effort. Likewise, critical information on the safety of siRNA-loaded nanoparticles in regards to injection/administration, clearance route, and potential immune response in long-lasting and sustained release platforms are still unclear. Strategies for diminishing the risks of nanotoxicity, and the methodology for evaluating these strategies need to be carefully designed. Most importantly, the lack of fast and inexpensive screening tools for intermediate and final adducts of functional NP-RNA complexes using pre-clinical animal models has greatly hampered clinical translation. Before these issues can be addressed, however, there is still much to be done in terms of biological barriers at the organismic, tissue, and cellular levels. Overcoming these challenges will require improved understanding of the fundamental aspects of nanomaterial interactions with biological systems, research that is underway world-wide.

4. Expert Opinion

RNAi has the potential to revolutionize treatment of a broad range of diseases, particularly “undruggable” targets. We currently have a diverse set of inorganic nanocarriers for siRNA delivery that are effective both *in vitro* and *in vivo*. Each of these approaches, however, currently have limitations and challenges that requires further optimization to overcome the barriers present for effective therapeutics.

Toxicology is an important issue, with inorganic NPs generating new and unpredicted behaviors. Most studies of the biocompatibility of inorganic NPs were evaluated using a simple cytotoxicity evaluation; however, detailed toxicological evaluation, such as cell membrane damage, oxidative stress, genotoxicity, are required for predicting long-term effects of NP exposure.

A key challenge for siRNA therapeutics is getting the material efficiently to where it needs to be. This issue is multi-scale, with hurdles at the organismic, tissue, and cellular level. On the organismic level, pharmacokinetics (PK) is an important concern. Effective nanoparticle vectors should minimize the recognition and clearance by the reticuloendothelial system (RES) after systemic administration. Grafting non-interacting functional groups, such as PEG and zwitterionic entities, have proved to be efficient at decreasing NP clearance by RES and increase *in vivo* circulation. However, these methods also decrease the siRNA delivery efficiency due the PEG shielding of the NPs or by decreasing the intracellular release of siRNA. Clearly, new chemistries for preventing serum protein adsorption onto NPs will improve the pharmacokinetics of NPs, and reduce the NP uptake by RES.

Biodistribution is an issue that will be helped but not solved by non-fouling coatings. The conjugation of targeted ligands to inorganic NPs enhanced the accumulation of siRNA at the disease sites, in particular, tumors. However, the decoration of target ligands on each type of NPs requires careful case-by-case validation for enhanced siRNA delivery. In addition, the

conjugation of targeted ligands to NPs may also alter the PK and stability of the vectors, complicating data interpretation of *in vivo* studies.

Perhaps the greatest hurdle for siRNA therapeutics, however, is avoiding endosomal entrapment and subsequent degradation. This is a crucial issue, as currently delivery to the cytosol is generally remarkably inefficient, with consequent off-target effects arising from the large doses of vector required for knockdown efficacy. Future design of inorganic NPs requires careful consideration and accurate optimization of NP chemical functionalities to improve cytosolic siRNA delivery and avoid off-target effects. For example, installing new functionality onto NPs to modulate siRNA release, using the disease microenvironment or characteristic disease types and stages, could deliver siRNA more efficiently. In addition, integrating optical or other tracking signals to NPs provides new approaches to evaluate the colloidal and surface ligand stability of NPs *in vivo*, offering feedback for optimizing the design of the nanoparticles to further improve the delivery. Additionally, chemically modified siRNA has exhibited higher RNA stability and reduced off-target effects compared to traditional unmodified siRNA. As a result NPs that can compensate for the new biophysical structure and biological function of modified siRNA are highly desirable. Finally, to apply these next-generation inorganic NPs in clinical settings, their ‘negative’ effects, such as the nanotoxicity and immunogenicity, should be carefully inspected. A comprehensive study of liver, inflammatory, hematologic, serologic, and kidney toxicity should be determined and correlated to NP structure.

Taken together, the tools that are available for achievements synthesis and surface engineering of inorganic NPs make these systems promising platforms for the very challenging yet rewarding goal of RNAi therapeutics.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

- 1•. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998; 391(6669):806–11. This is the first report of RNAi discovered in *Caenorhabditis elegans*. [PubMed: 9486653]
2. Leung RK, Whittaker PA. RNA interference: from gene silencing to gene-specific therapeutics. *Pharmacol Ther*. 2005; 107(2):222–39. [PubMed: 15908010]
3. Aagaard L, Rossi JJ. RNAi therapeutics: principles, prospects and challenges. *Adv Drug Deliv Rev*. 2007; 59(2–3):75–86. [PubMed: 17449137]
- 4••. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. *Nat Rev Drug Discov*. 2009; 8(2):129–38. An excellent review summarizes the challenges and barriers of non-viral siRNA delivery. [PubMed: 19180106]
5. Dykxhoorn DM, Palliser D, Lieberman J. The silent treatment: siRNAs as small molecule drugs. *Gene Ther*. 2006; 13(6):541–52. [PubMed: 16397510]
6. Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. *Chem Biol*. 2012; 19(1):60–71. [PubMed: 22284355]
7. Roth L. First evidence of RNA interference mechanism induced in human: getting closer to a cure? *Cell Adh Migr*. 2011; 5(1):1–3. [PubMed: 20890128]
8. He S, Zhang D, Cheng F, Gong F, Guo Y. Applications of RNA interference in cancer therapeutics as a powerful tool for suppressing gene expression. *Mol Biol Rep*. 2009; 36(8):2153–63. [PubMed: 19117119]

9. Oh Y-K, Park TG. siRNA delivery systems for cancer treatment. *Adv Drug Deliv Rev.* 2009; 61(10): 850–62. [PubMed: 19422869]
10. Schifflers RM, Ansari A, Xu J, Zhou Q, Tang Q, Storm G, et al. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. *Nucleic Acids Res.* 2004; 32(19):e149. [PubMed: 15520458]
- 11•. Yuan X, Naguib S, Wu Z. Recent advances of siRNA delivery by nanoparticles. *Expert Opin Drug Deliv.* 2011; 8(4):521–36. A review of nanoparticle based siRNA delivery. [PubMed: 21413903]
12. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. *Nat Rev Genet.* 2014; 15(8):541–55. [PubMed: 25022906]
13. Hiruta C, Toyota K, Miyakawa H, Ogino Y, Miyagawa S, Tatarazako N, et al. Development of a microinjection system for RNA interference in the water flea *Daphnia pulex*. *BMC Biotechnol.* 2013; 13(1):96. [PubMed: 24188141]
14. Tomar RS, Matta H, Chaudhary PM. Use of adeno-associated viral vector for delivery of small interfering RNA. *Oncogene.* 2003; 22(36):5712–15. [PubMed: 12944921]
15. Barton GM, Medzhitov R. Retroviral delivery of small interfering RNA into primary cells. *Proc Natl Acad Sci USA.* 2002; 99(23):14943–45. [PubMed: 12417750]
16. Waehler R, Russell SJ, Curiel DT. Engineering targeted viral vectors for gene delivery. *Nat Rev Genet.* 2007; 8(8):573–87. [PubMed: 17607305]
17. Wagner E. Polymers for siRNA Delivery: Inspired by Viruses to be Targeted, Dynamic, and Precise. *Acc Chem Res.* 2012; 45(7):1005–13. [PubMed: 22191535]
18. Wang M, Alberti K, Varone A, Pouli D, Georgakoudi I, Xu Q. Enhanced intracellular siRNA delivery using bioreducible lipid-like nanoparticles. *Adv Health Mater.* 2014; 3(9):1398–403.
19. Galaway FA, Stockley PG. MS2 viruslike particles: a robust, semisynthetic targeted drug delivery platform. *Mol Pharm.* 2013; 10(1):59–68. [PubMed: 23110441]
20. van Asbeck AH, Beyerle A, McNeill H, Bovee-Geurts PHM, Lindberg S, Verdurmen WPR, et al. Molecular parameters of siRNA–cell penetrating peptide nanocomplexes for efficient cellular delivery. *ACS Nano.* 2013; 7(5):3797–807. [PubMed: 23600610]
- 21•. Conde J, Ambrosone A, Hernandez Y, Tian F, McCully M, Berry CC, et al. 15 years on siRNA delivery: Beyond the state-of-the-art on inorganic nanoparticles for RNAi therapeutics. *Nano Today.* 2015; A very recent review of inorganic nanoparticle for siRNA delivery. doi: 10.1016/j.nantod.2015.06.008
22. Dominska M, Dykxhoorn DM. Breaking down the barriers: siRNA delivery and endosome escape. *J Cell Sci.* 2010; 123(8):1183–89. [PubMed: 20356929]
23. Ma D. Enhancing endosomal escape for nanoparticle mediated siRNA delivery. *Nanoscale.* 2014; 6(12):6415–25. [PubMed: 24837409]
24. Sokolova V, Epple M. Inorganic nanoparticles as carriers of nucleic acids into cells. *Angew Chem Int Ed.* 2008; 47(8):1382–95.
25. Zhang L, Li Y, Yu JC. Chemical modification of inorganic nanostructures for targeted and controlled drug delivery in cancer treatment. *J Mater Chem B.* 2014; 2(5):452–70.
26. Zhao MX, Zeng EZ, Zhu BJ. The biological applications of inorganic nanoparticle drug carriers. *Chem Nano Mat.* 2015; 1(2):82–91.
- 27•. Ding Y, Jiang Z, Saha K, Kim CS, Kim ST, Landis RF, et al. Gold nanoparticles for nucleic acid delivery. *Mol Ther.* 2014; 22(6):1075–83. A review of gold nanoparticle based gene delivery. [PubMed: 24599278]
28. Pissuwan D, Niidome T, Cortie MB. The forthcoming applications of gold nanoparticles in drug and gene delivery systems. *J Control Release.* 2011; 149(1):65–71. [PubMed: 20004222]
29. Han G, Ghosh P, Rotello VM. Functionalized gold nanoparticles for drug delivery. *Nanomedicine.* 2007; 2(1):113–23. [PubMed: 17716197]
30. Ho D, Sun X, Sun S. Monodisperse magnetic nanoparticles for theranostic applications. *Acc Chem Res.* 2011; 44(10):875–82. [PubMed: 21661754]
31. Lim E-K, Kim T, Paik S, Haam S, Huh Y-M, Lee K. Nanomaterials for theranostics: recent advances and future challenges. *Chem Rev.* 2015; 115(1):327–94. [PubMed: 25423180]

32. Rana S, Bajaj A, Mout R, Rotello VM. Monolayer coated gold nanoparticles for delivery applications. *Adv Drug Deliv Rev.* 2012; 64(2):200–16. [PubMed: 21925556]
33. Oishi M, Nakaogami J, Ishii T, Nagasaki Y. Smart PEGylated gold nanoparticles for the cytoplasmic delivery of siRNA to induce enhanced gene silencing. *Chem Lett.* 2006; 35(9):1046–47.
34. Giljohann DA, Seferos DS, Prigodich AE, Patel PC, Mirkin CA. Gene regulation with polyvalent siRNA-nanoparticle conjugates. *J Am Chem Soc.* 2009; 131(6):2072–3. Spherical nucleic acid nanoparticle conjugates for siRNA delivery and gene silencing. [PubMed: 19170493]
35. Zheng D, Giljohann DA, Chen DL, Massich MD, Wang XQ, Iordanov H, et al. Topical delivery of siRNA-based spherical nucleic acid nanoparticle conjugates for gene regulation. *Proc Natl Acad Sci USA.* 2012; 109(30):11975–80. [PubMed: 22773805]
36. Jensen SA, Day ES, Ko CH, Hurley LA, Luciano JP, Kouri FM, et al. Spherical nucleic Acid nanoparticle conjugates as an rNAi-based therapy for glioblastoma. *Sci Transl Med.* 2013; 5(209):209ra152.
37. Randeria PS, Seeger MA, Wang XQ, Wilson H, Shipp D, Mirkin CA, et al. siRNA-based spherical nucleic acids reverse impaired wound healing in diabetic mice by ganglioside GM3 synthase knockdown. *Proc Natl Acad Sci USA.* 2015; 112(18):5573–8. [PubMed: 25902507]
38. Huang X, Hu Q, Braun GB, Pallaoro A, Morales DP, Zasadzinski J, et al. Light-activated RNA interference in human embryonic stem cells. *Biomaterials.* 2015; 63:70–9. [PubMed: 26086448]
39. Lee JS, Green JJ, Love KT, Sunshine J, Langer R, Anderson DG. Gold, poly(beta-amino ester) nanoparticles for small interfering RNA delivery. *Nano Lett.* 2009; 9(6):2402–6. [PubMed: 19422265]
40. Conde J, Tian F, Hernandez Y, Bao C, Cui D, Janssen KP, et al. In vivo tumor targeting via nanoparticle-mediated therapeutic siRNA coupled to inflammatory response in lung cancer mouse models. *Biomaterials.* 2013; 34(31):7744–53. [PubMed: 23850099]
41. Son S, Nam J, Kim J, Kim S, Kim WJ. i-motif-driven Au nanomachines in programmed siRNA delivery for gene-silencing and photothermal ablation. *ACS Nano.* 2014; 8(6):5574–84. [PubMed: 24869928]
42. Kim ST, Chompoosor A, Yeh YC, Agasti SS, Solfiell DJ, Rotello VM. Dendronized gold nanoparticles for siRNA delivery. *Small.* 2012; 8(21):3253–6. [PubMed: 22887809]
43. Lee Y, Lee SH, Kim JS, Maruyama A, Chen X, Park TG. Controlled synthesis of PEI-coated gold nanoparticles using reductive catechol chemistry for siRNA delivery. *J Control Release.* 2011; 155(1):3–10. [PubMed: 20869409]
44. Song W-J, Du J-Z, Sun T-M, Zhang P-Z, Wang J. Gold nanoparticles capped with polyethyleneimine for enhanced siRNA delivery. *Small.* 2010; 6(2):239–46. [PubMed: 19924738]
45. Elbakry A, Zaky A, Liebl R, Rachel R, Goepferich A, Breunig M. Layer-by-layer assembled gold nanoparticles for siRNA delivery. *Nano Lett.* 2009; 9(5):2059–64. [PubMed: 19331425]
46. Lee MY, Park SJ, Park K, Kim KS, Lee H, Hahn SK. Target-specific gene silencing of layer-by-layer assembled gold-cysteamine/siRNA/PEI/HA nanocomplex. *ACS Nano.* 2011; 5(8):6138–47. [PubMed: 21739990]
47. Lee SK, Han MS, Asokan S, Tung CH. Effective gene silencing by multilayered siRNA-coated gold nanoparticles. *Small.* 2011; 7(3):364–70. [PubMed: 21294265]
48. Lee SK, Tung C-H. A fabricated siRNA nanoparticle for ultralong gene silencing *in vivo*. *Adv Funct Mater.* 2013; 23(28):3488–93. [PubMed: 24999314]
49. Guo S, Huang Y, Jiang Q, Sun Y, Deng L, Liang Z, et al. Enhanced gene delivery and siRNA silencing by gold nanoparticles coated with charge-reversal polyelectrolyte. *ACS Nano.* 2010; 4(9):5505–11. pH-responsive LBL self-assembled AuNP/siRNA nanoparticle. [PubMed: 20707386]
50. Han L, Zhao J, Zhang X, Cao W, Hu X, Zou G, et al. Enhanced siRNA delivery and silencing gold-chitosan nanosystem with surface charge-reversal polymer assembly and good biocompatibility. *ACS Nano.* 2012; 6(8):7340–51. [PubMed: 22838646]
51. Gilleron J, Querbes W, Zeigerer A, Borodovsky A, Marsico G, Schubert U, et al. Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape. *Nat Biotechnol.* 2013; 31(7):638–46. [PubMed: 23792630]

52. Ding Y, Wang Y, Zhou J, Gu X, Wang W, Liu C, et al. Direct cytosolic siRNA delivery by reconstituted high density lipoprotein for target-specific therapy of tumor angiogenesis. *Biomaterials*. 2014; 35(25):7214–27. [PubMed: 24875759]
53. Benjaminsen RV, Mattheijer MA, Henriksen JR, Moghimi SM, Andresen TL. The possible proton sponge effect of polyethylenimine (PEI) does not include change in lysosomal pH. *Mol Ther*. 2013; 21(1):149–57. [PubMed: 23032976]
54. Hatakeyama H, Ito E, Akita H, Oishi M, Nagasaki Y, Futaki S, et al. A pH-sensitive fusogenic peptide facilitates endosomal escape and greatly enhances the gene silencing of siRNA-containing nanoparticles *in vitro* and *in vivo*. *J Control Release*. 2009; 139(2):127–32. [PubMed: 19540888]
55. Li X, Chen Y, Wang M, Ma Y, Xia W, Gu H. A mesoporous silica nanoparticle-PEI-fusogenic peptide system for siRNA delivery in cancer therapy. *Biomaterials*. 2013; 34(4):1391–401. [PubMed: 23164421]
56. Lu W, Zhang G, Zhang R, Flores LG 2nd, Huang Q, Gelovani JG, et al. Tumor site-specific silencing of NF-kappaB p65 by targeted hollow gold nanosphere-mediated photothermal transfection. *Cancer Res*. 2010; 70(8):3177–88. [PubMed: 20388791]
57. Jiang Y, Tang R, Duncan B, Jiang Z, Yan B, Mout R, et al. Direct cytosolic delivery of siRNA using nanoparticle-stabilized nanocapsules. *Angew Chem Int Ed*. 2015; 54(2):506–10. Direct cytosolic siRNA delivery using gold nanoparticle stabilized nanocapsule.
58. Lee JH, Lee K, Moon SH, Lee Y, Park TG, Cheon J. All-in-one target-cell-specific magnetic nanoparticles for simultaneous molecular imaging and siRNA delivery. *Angew Chem Int Ed*. 2009; 48(23):4174–9.
59. Kumar M, Yigit M, Dai G, Moore A, Medarova Z. Image-guided breast tumor therapy Using a small interfering RNA nanodrug. *Cancer Res*. 2010; 70(19):7553–61. [PubMed: 20702603]
60. Liu G, Xie J, Zhang F, Wang Z, Luo K, Zhu L, et al. N-Alkyl-PEI-functionalized iron oxide nanoclusters for efficient siRNA delivery. *Small*. 2011; 7(19):2742–9. [PubMed: 21861295]
61. Park JW, Bae KH, Kim C, Park TG. Clustered magnetite nanocrystals cross-linked with PEI for efficient siRNA delivery. *Biomacromolecules*. 2011; 12(2):457–65. [PubMed: 21190334]
62. Agrawal A, Min D-H, Singh N, Zhu H, Birjiniuk A, von Maltzahn G, et al. Functional delivery of siRNA in mice using dendriworms. *ACS Nano*. 2009; 3(9):2495–504. [PubMed: 19673534]
63. Jiang S, Eltoukhy AA, Love KT, Langer R, Anderson DG. Lipidoid-coated iron oxide nanoparticles for efficient DNA and siRNA delivery. *Nano Lett*. 2013; 13(3):1059–64. [PubMed: 23394319]
64. Namiki Y, Namiki T, Yoshida H, Ishii Y, Tsubota A, Koido S, et al. A novel magnetic crystal-lipid nanostructure for magnetically guided in vivo gene delivery. *Nat Nanotechnol*. 2009; 4(9):598–606. [PubMed: 19734934]
65. Slowing II, Vivero-Escoto JL, Wu C-W, Lin VSY. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. *Adv Drug Deliv Rev*. 2008; 60(11):1278–88. [PubMed: 18514969]
66. Xia T, Kovochich M, Liong M, Meng H, Kabehie S, George S, et al. Polyethyleneimine coating enhances the cellular uptake of mesoporous silica nanoparticles and allows safe delivery of siRNA and DNA constructs. *ACS Nano*. 2009; 3(10):3273–86. [PubMed: 19739605]
67. Bhattarai S, Muthuswamy E, Wani A, Brichacek M, Castañeda A, Brock S, et al. Enhanced gene and siRNA delivery by polycation-modified mesoporous silica nanoparticles with chloroquine. *Pharm Res*. 2010; 27(12):2556–68. [PubMed: 20730557]
68. Hartono SB, Gu W, Kleitz F, Liu J, He L, Middelberg APJ, et al. Poly-L-lysine functionalized large pore cubic mesostructured silica nanoparticles as biocompatible carriers for gene delivery. *ACS Nano*. 2012; 6(3):2104–17. [PubMed: 22385282]
69. Kapilov-Buchman Y, Lellouche E, Michaeli S, Lellouche JP. Unique surface modification of silica nanoparticles with polyethylenimine (PEI) for siRNA delivery using cerium cation coordination chemistry. *Bioconjug Chem*. 2015; 26(5):880–9. [PubMed: 25830668]
70. Ngamcherdtrakul W, Morry J, Gu S, Castro DJ, Goodyear SM, Sangvanich T, et al. Cationic polymer modified mesoporous silica nanoparticles for targeted siRNA delivery to HER2+ breast cancer. *Adv Funct Mater*. 2015; 25(18):2646–59. [PubMed: 26097445]

71. Chen AM, Zhang M, Wei D, Stueber D, Taratula O, Minko T, et al. Co-delivery of doxorubicin and Bcl-2 siRNA by mesoporous silica nanoparticles enhances the efficacy of chemotherapy in multidrug-resistant cancer cells. *Small*. 2009; 5(23):2673–7. [PubMed: 19780069]
72. Ma X, Zhao Y, Ng KW, Zhao Y. Integrated hollow mesoporous silica nanoparticles for target drug/siRNA co-Delivery. *Chem J Eur*. 2013; 19(46):15593–603.
73. Meng H, Mai WX, Zhang H, Xue M, Xia T, Lin S, et al. Codelivery of an optimal drug/siRNA combination using mesoporous silica nanoparticles to overcome drug resistance in breast cancer in vitro and in vivo. *ACS Nano*. 2013; 7(2):994–1005. Silica nanoparticle for the co-delivery of siRNA and chemotherapy drug. [PubMed: 23289892]
74. Roy I, Mitra S, Maitra A, Mozumdar S. Calcium phosphate nanoparticles as novel non-viral vectors for targeted gene delivery. *Int J Pharm*. 2003; 250(1):25–33. [PubMed: 12480270]
75. Pittella F, Zhang M, Lee Y, Kim HJ, Tockary T, Osada K, et al. Enhanced endosomal escape of siRNA-incorporating hybrid nanoparticles from calcium phosphate and PEG-block charge-conversional polymer for efficient gene knockdown with negligible cytotoxicity. *Biomaterials*. 2011; 32(11):3106–14. [PubMed: 21272932]
76. Lee MS, Lee JE, Byun E, Kim NW, Lee K, Lee H, et al. Target-specific delivery of siRNA by stabilized calcium phosphate nanoparticles using dopa–hyaluronic acid conjugate. *J Control Release*. 2014; 192:122–30. [PubMed: 24995950]
77. Li J, Yang Y, Huang L. Calcium phosphate nanoparticles with an asymmetric lipid bilayer coating for siRNA delivery to the tumor. *J Control Release*. 2012; 158(1):108–14. [PubMed: 22056915]

Article highlights

- RNAi has the potential to revolutionize the treatment of a broad range of diseases, particularly conventionally “undruggable” targets.
- Several challenges exist in developing nanocarriers for effective siRNA delivery, the greatest of which are avoiding endosomal entrapment and the subsequent degradation of siRNA.
- Inorganic nanoparticles provide a useful platform for the engineering of RNAi therapeutics for in vitro and in vivo applications.
- The physicochemical properties of NPs determine the loading of siRNA, intracellular uptake, endosomal escape, and targeted delivery of siRNA to disease cells and tissues.
- Effective delivery of siRNA at the organismic and cellular levels, coupled with the low immunogenic response of inorganic NPs, will facilitate the clinical translation of RNAi treatments for genetic diseases.

Figure 1.
Cellular mechanism of RNA interference. **Reprinted with permission from [4].**

Figure 2.

(a) SNAs are 13-nm gold cores densely functionalized with highly oriented, thiolated siRNA duplexes that target GM3S. The SNA surface is passivated with oligoethylene glycol for colloidal stability. **Reprinted with permission from ref. 37. Copyright 2015 National Academy of Sciences.** (b) siRNA delivery using dendronized AuNPs. Chemical structure of G2-AuNP and schematic illustration of G2-AuNP/ β -gal-siRNA complexation and transfection into SVR-bag4 cells. **Reprinted with permission from ref. [42]** (c) Enhanced intracellular payload release at endosome by pH-dependent charge-reversal polyelectrolyte on gold nanoparticles. **Reprinted with permission from [49].**

Figure 3.

(a) Schematic illustration of the proton sponge effect leading to siRNA release into the cytosol. **Adapted with permission from [27].** (b) NPSC/siRNA components and proposed mechanism of cytosolic siRNA delivery. (c) Live cell imaging of rapid Cy3-siRNA release into the cytosol of HeLa cell by NPSCs. “0 min” label represents the starting point of release. Scale bars: 20 μm . (d) Efficient knockdown of deGFP using NPSC/sideGFP with 40nM siRNA concentration after 48 h delivery. **Adapted with permission from ref. 57.** Copyright 2015 John Wiley and Sons.

Figure 4.

(a) Synthetic scheme for MnMEIO-siGFP-Cy5/PEG-RGD. SPDP, which forms disulfide bonds, was used as a linker. **Reprinted with permission from [58]** (b) Schematic diagram of a co-delivery system based on MSNs to deliver Dox and Bcl-2-targeted siRNA simultaneously to A2780/AD human ovarian cancer cells for enhanced chemotherapy efficacy. **Reprinted with permission from [71].**

Figure 5. Schematic illustration of the formation and cellular uptake of CAP/siRNA/dopa-HA nanoparticles. **Reprinted with permission from [76].**

Table 1

Examples of inorganic nanoparticle-based *in vitro* and *in vivo* siRNA delivery systems.

Nanoparticle	Surface chemistry	Target gene	Cell line/tumor model	Reference
AuNP-siRNA conjugated	thiol-siRNA	luciferase	hepatoma HuH-7 cells	33
	thiol-siRNA	Bcl2L12	U87MG xenograft	36
	thiol-siRNA	GM3S	diabetic mice	37
	TAT peptide and thiol-siRNA	Oct4	human embryonic stem cells (hESC)	38
	Poly (β -amino ester)	luciferase	HeLa cells	39
	thiol-siRNA and Arg-Gly-Asp (RGD)	c-myc, luciferase	CMT/167 lung tumor mice	40
	<i>i</i> -motif DNA and thiol-siRNA	PLK1	NIH3T3 cells	41
AuNPs	Dendritic triethylenetetramine (TETA)	β -galactosidase	SVR-bag4 cells	42
	catechol-conjugated PEI	GFP and PLK-1	MDA-MB-435 cancer cell	43
	cysteamine (CM)/hyaluronic acid(HA)/PEI	ApoB	mouse liver	46
	siRNA/PLL multilayer	luciferase	MDA-MB-231	47-48
	PEI/PAHCit/PEI/MUA	MDR1	HeLa cells	49
Hollow AuNPs	Thiol-siRNA	NF-KB p65	HeLa xenograft	56
NPSC	Arginine	GFP	HEK 293 cells	57
Magnetic NPs	serum albumin (BSA)	GFP	MDA-MB-435 cells	58
	Cy5.5 and uMUC-1 targeting peptide	BIRC5	MDA-MB-435 and A549 cells	59
	Alkylated PEI	luciferase	fLuc-4T1 xenograft model	60
	dendrimer-conjugated	EGFR	Mouse brain	62
	Lipidoid C14–200	luciferase	HeLa cells	63
	lipid	EGFR	NUGC-4 and MKN-74 xenograft	64
Mesoporous Silica NPs	PEI	GFP	HEPA-1 liver cancer cells	66
	PEI and fusogenic KALA peptide	VEGF	A549 xenograft	55
	Ce ³⁺ - crosslinked bPEI	luciferase	U2OS human osteosarcoma cell	69
	anti-HER2 monoclonal antibody/PEI	HER2	HER2-positive BT474 cells HCC1954 tumors bearing mice	70
	PAMAM dendrimer/ folic acid	Bcl-2	A2780/AD human ovarian cancer cells	71
	co-delivery of doxorubicin and siRNA	P-gp	MCF-7/MDR xenograft	73
Calcium phosphate NPs	PEG-PMA polymer	luciferase	HEK 293 cells	75
	DOPA conjugated hyaluronic acid (HA)	luciferase	HT-29 xenograft	76
	DOPA/DOTAP or DOPA/DOPC	luciferase	H460 xenograft	77

Table 2

Comparison of organic and inorganic NPs-based siRNA delivery systems.

	Advantages	Disadvantages
Organic NPs-based siRNA carrier	Low cost/scalable Easy functionalization Enables passive/active targeting Controllable drug release Moderate siRNA protection Biocompatible and biodegradable A few of clinical trails	Short shelf life Relative low stability Biocompatibility issues Low immunogenicity Rapid clearance (uptake by RES) Batch to batch variation Limited efficiency
Inorganic NPs-based siRNA carrier	Long shelf life High stability (over wide a range of temperature and pH) Tunable size and shape Optical properties (as imaging agents) Remote controlled release (Magnetic NPs) Easy functionalization Enables passive/active targeting High loading capacity Easy and rapid internalization Superior stability against enzymatic degradation	Poor biodegradability Toxicity issues (long-term safety) Poor scale up Batch to batch variation Limited efficiency Lack of clinical trails