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Engineering Functional Inorganic-Organic Hybrid System Advances in siRNA Therapeutics

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Abstract

Cancer treatment still faces a lot of obstacles such as tumor heterogeneity, drug resistance and systemic toxicities. Beyond the traditional treatment modalities, exploitation of RNA interference (RNAi) as an emerging approach has immense potential for treatment various gene-caused diseases including cancer. The last decade has witnessed enormous research and achievements focused on RNAi biotechnology. However, delivery of small interference RNA (siRNA) remains a key challenge in the development of clinical RNAi therapeutics. Indeed, the functional nanomaterials play an important role in the siRNA delivery, which could overcome a wide range of sequential physiological and biological obstacles. Nanomaterials-formulated siRNA systems have potential applications for protection of siRNA from degradation, improving the accumulation in the target tissues, enhancing the siRNA therapy and reducing the side effects. In this review, we explore and summarize the role of functional inorganic-organic hybrid systems involved in the siRNA therapeutic advancements. Additionally, we gather up the surface engineering strategies of hybrid systems to optimize for siRNA delivery. Major progress in the field of inorganic-organic hybrid platforms including metallic/non-metallic core modified with organic shell or further fabrication as the vectors for siRNA delivery is discussed to give credit to the interdisciplinary cooperation between chemistry, pharmacy, biology and medicine.

Graphical abstract

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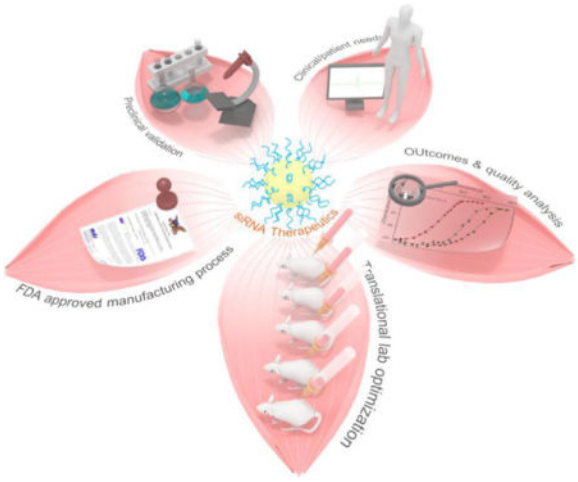
Conflict of Interest

The authors have declared that no conflicts of interest exist.

Beyond the traditional treatment modalities, RNA interference (RNAi) has immense potential for the treatment of various human diseases including cancer. Despite the great versatility of RNAi technology, which could down-regulate any protein in targeted cells and tissues, the major challenge for RNAi-based therapy is indeed the development of a delivery system. The engineering functional inorganic-organic hybrid vectors-formulated siRNA systems as an emerging approach to overcome many physiological and biological obstacles pave the way of successful, safe and efficient platform in clinical applications.



Keywords

Cancer; RNA interference; small interfering RNA; delivery; inorganic-organic hybrid nanoparticles; engineering strategies

1. Introduction

In spite of the huge advancement achieved in research, drug and technology development for cancer treatment during the past decades, cancer still is a major public health problem and is a main leading cause of death worldwide. Human cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. Additionally, human cancers consist of a mixed population of malignant cells, which carry multiple genetic mutations caused by external and internal factors, such as tobacco smoke, infectious organisms, unhealthy diet, inherited genetic burden, hormones, and immune conditions.[1] These factors may act together or in sequence to cause new cancer cases and deaths. As shown in Figure 1A, around 1.6 million new cases of cancer were diagnosed and about 35% cancer deaths in US per year during last five years. Additionally, more than 50% cancer patients die annually according to statistics from 2000 to 2011 in China (Fig. 1B).[2] Especially, there are 4.292 million new cases and 2.814 million cancer deaths in last year (Fig. 1C).[3, 4] Moreover, the number of new cases and deaths are 14.1 and 8.2 million in the world in 2012 (Fig. 1D).[5] Thus, cancer is a major issue to affect the healthcare. Currently, treatments include surgery, radiation, chemotherapy, hormone therapy, immune therapy, gene therapy and targeted therapy with their own limitations.[6] However, many of cancers are driver mutations causing tumor initiation, progression, metastasis and drug resistance that results in urgent need for

new technologies and strategies to fight cancer. Among the emerging technologies and anti-cancer therapeutics, small interfering RNA (siRNA) and RNA interference (RNAi) have been widely recognized to be capable of silencing many or perhaps all genes that results in modulation or targeted blockage of the biological processes, which are the defining hallmarks of cancer. Therefore, RNAi would pave the way for the cancer treatment as a promising biotechnology.[7, 8]

Since its discovery by Andrew Fire and Craig Mello, it was demonstrated in 1998 that long double-stranded RNA (dsRNA) mixtures were 10-100-folds more efficient at causing gene silencing than the single strand RNAs in *Caenorhabditis elegans* (Fig. 2A).[9] Four years later, Science named RNAi "technology of the year". Fire and Mello were awarded jointly the Nobel Prize in Physiology or Medicine for their discovery of RNAi-gene silencing by siRNA in 2006 (Fig. 2A). [10] Then, the therapeutics based on siRNA as a potent drug candidate for gene regulation came when Elbashir and co-workers proved that the synthetic siRNA enabled sequence-specific gene down-regulation in a mammalian cell line. [11] Meanwhile, nanotechnology plays an important role in drug delivery including siRNA therapeutics, e.g. the first targeted nanoparticle-based siRNA therapeutics delivery in human cancer patients in 2010 (Fig. 2A).[12–14] Indeed, a tremendous amount of effort has been put into development of siRNA cancer therapeutics over the last 20 years, and great progress has been achieved in research and development, both in academics and pharmaceutical industry. Although, in one period the big pharmaceutical factories closed the siRNA-related projects due to the disappointing results.[15] Over the last 5 years, siRNA therapeutics has achieved a rapid development in fighting various diseases including cancer, which is indicated by over 30% cases that have already achieved Phase 2 or Phase 3 clinical trials (Table 1). Moreover, the leading regional markets of RNAi not only set up in developed countries, but also in developing countries (Fig. 2B). However, most of the leading companies of RNAi are in USA or EU5 ranging from 2015 to 2025 (Fig. 2C) (https://www.researchandmarkets.com/research/td7m2r/rnai). This trend indicates that the RNAi would be a strong promise therapy for treatment of various human diseases in the future. Such big progresses have brought new avenue for the siRNA therapeutics in clinical trials. Additionally, these achievements laid the foundations to employ RNAi as a key tool for next generation cancer drugs.

2. Biochemical properties of siRNA and RNAi

siRNA is a typical short/small double-stranded RNA molecules, approximately 21 nucleotides in length, and about 19 bp of the core region of siRNA (Fig. 3A) with the ability to mobilize the RNAi pathway.[35] The interference effect has revealed immense potential for regulate the diseases caused by the overexpression or mutation of gene including cancer, genetic disorder and other diseases because of sequence-specific gene silencing. The structure is also well defined, siRNAs have phosphorylated 5' ends and hydroxylated 3' ends with two overhanging nucleotides. Additionally, naked siRNA has a large molecular weight (14 kD) and negative charge (polyanionic nature of the phosphate charges). Once the long siRNA or siRNA introduced inside the cytoplasm, the enzyme endoribonuclease Dicer would cleave the long siRNA into fragments siRNA (21 bp), then siRNA is incorporated into RNA inducing silencing complex (RISC), which consists of the Argonaute (Ago) protein as one of its main components, that results in splitting of each double-stranded

siRNA into the passenger (sense) strand and the guide (antisense) strand. The sense strand is degraded, whereas the antisense strand is incorporated into RISC as an activated RISC complexes form that further direct the specificity of the target mRNA recognition via complementary base pairing. Later, Ago-2 catalyzes cleavage of the target mRNA thereby triggering the protein down-regulation and gene silencing (Fig. 3B).[35] Comparing to the anticancer chemotherapeutic drugs, siRNA possesses a number of advantages due to its specific mechanism. The benefits of siRNA as a potential anticancer drug is the strongest supported by its effective inhibition of the any interest target mRNA or mutated gene in any type of cancer cells. Moreover, the synthesis of siRNA is relatively easy and its production costs less.

3. Bio-barriers of siRNA therapeutics

In spite of advancement in preclinical and clinical studies, no product has been launched into the market, because there are still many challenges to be overcome for siRNA clinical therapeutics. As well known, siRNA molecules have unfavorable physicochemical properties, such as negative charges, large molecule weight and size, and instability that impede the access through the cell membrane. Serum endonuclease easily degrades naked siRNA then easily eliminated through glomerular filtration. This implies that the double-stranded siRNA molecules have a very short half-life (10 min) and very low stability. This issue can be overcome by chemical modifications of the RNA backbone and by embedding the siRNA into nano-carriers. [35]In order to have effect, siRNA needs to arrive in the cytoplasm of the cancer cell. From the region of injection (if systemic delivery is used) to the site of action, the siRNA molecule faces many biological obstacles. Challenges that need to be overcome are safety, stability and effective delivery.

From the blood circulation to the tumor cell, the RNAi and/or its transport mechanism needs to additionally overcome biobarriers. Biobarriers are biological surfaces (epithelia, endothelia, cellular, nuclear, endosomal membranes, etc.), which can be multi-cellular and/or multi-compartmental. These are elements of separation within defined compartments (e.g. vascular, cytoplasmic, stromal, etc.) and between different compartments. In the delivery of nanoparticles to tumor sites, biobarriers comprise all “barriers” that a nanoparticle needs to cross in order to reach the target cell. Using systemic administration (e.g. intravenous injection of nanoparticles), the biobarriers that need to be addressed are: 1) Enzymatic degradation; 2) Reticulo-Endothelial (RE) organs sequestration and phagocytes; 3) Tumor interstitium; 4) Crossing the cellular membrane; 5) Endosomal escape in order to prevent degradation in lysosomes and/or excretion from the cell (Fig. 4).[36] An adequate negotiation of these barriers is therefore necessary in order to successfully deliver the siRNA into the cell obtaining a sufficient therapeutic index.

So far, the major barriers that prevent siRNA therapeutics from reaching the market are poor resistance to enzymes, such as RNase, short biological half-life, lack of cell/tissue targeting, ineffective cellular internalization, complicated escape from endosomes/lysosomes, toxicity and other related side effects and difficulties (Fig. 4).[37] Therefore, the successful delivery of siRNA to the targeted cell/organs is the most important issue for treatment of cancer in the clinical application.

Therefore, to address these issues, a special system for siRNA delivery is needed. Delivery systems used nowadays can be categorized into physical methods, conjugation methods, methods with natural carriers, such as viruses and bacteria, and non-viral carrier methods.[38] The use of viral vectors for the delivery of siRNA tends to be avoided because of insertional mutagenesis and immunogenicity problems that may arise due to the nature of the carrier. A new, non-viral way to overcome biobarriers, the added capability with respect to traditional cancer treatment methods of multi-functionality and specific cell targeting, has been proposed by nanomedicine research – nanoparticles.

Notably, the rational design of siRNA carriers is an important consideration because the use of high quantities of carriers can result in toxicity as a consequence of poor metabolism and elimination of the carriers. A great number of systems were reported for siRNA delivery based on biomaterial platforms, such as lipid, polymer, and nanoparticle.[39–41] Here we put the concept of the inorganic-organic hybrid nanoparticles-based siRNA therapeutics. Additionally, we review the engineering strategies of inorganic-organic hybrid systems and examine the details of metallic and non-metallic core hybrid with organic moieties for siRNA delivery. Finally, we summarize the advantages of such hybrid systems for paving the way for the next generation siRNA therapy.

4. Surface Engineering Strategies of Inorganic-Organic Hybrid Nanoparticles

The application of nanomaterials in biomedical areas shows great promise and inorganic nanomaterials attract a particular interest for bioimaging, diagnosis, drug delivery and therapy applications. As regards to the above-mentioned siRNA delivery, inorganic nanomaterials also exhibit great potential to reveal the mechanism of siRNA transportation process and subsequently to construct well-designed vectors to circumvent the biological barriers of siRNA delivery. To endow inorganic nanostructures with siRNA transportation capacity, surface engineering with organic ligands should firstly be carried out. Many strategies have been developed for the surface engineering of inorganic nanomaterials, and they can be divided into the following categories: covalent ligand conjugation, amphiphilic polymer assembly, electrostatic layer-by-layer assembly, *in-situ* ligand coating during synthesis, host-guest supramolecular ligand self-assembly, and lipid shell coating (Fig. 5).

4.1. Covalent ligand conjugation

The surface chemistry of different kinds of inorganic nanoparticles has been extensively explored for surface engineering via covalent ligand conjugation. Several kinds of anchoring groups have been developed for ligand conjugation, such as thiol, carboxyl, phosphate terminal ligands (Table 2). Thiols have been widely used as an anchoring unit to fabricate inorganic nanoparticles, mainly metallic nanoparticles. Thiols have very high affinity with many metal ions, such as Au, Ag, Cu, Zn, etc. via coordination interaction. Thereby, thiol-containing cationic ligands can be used for binding to nanoparticle surface to generate hybrid nanoparticles for siRNA delivery. Alternatively, nanoparitlces can be bond with heterobifunctional ligand containing thiol and another functional group, then further modified with other positively charged ligands. Rotello et al. synthesized three thiol-

containing dendron ligands with triethylenetetramine (TETA) terminal groups of different generations.[42] These ligands were used to modify gold nanoparticles (AuNPs) through Au-SH coordinate binding. The as-prepared hybrid nanoparticles are cationic and resist aggregation, which were shown to be effective nanocarriers for siRNA delivery. Similar to thiol ligands, disulfide terminal group containing ligands also can bind with nanoparticle through S-metal bond. In addition to AuNPs, a lot of other inorganic nanoparticles can be modified with thiol or disulfide ligands, such as silver nanoparticles (AgNPs), quantum dots (QDs), CuS nanoparticles, and so on. Hydrophobic iron oxide nanoparticles (IONPs) also could be functionalized through covalent ligand exchange strategy. Xu et al. reported that bidentate ligand, dopamine, can coordinate to the surface of IONPs owing to improved orbital overlap of the five-membered ring and a reduced steric effect on the iron complex.[43] The resulting IONPs showed excellent stability in physiological conditions. For non-metal inorganic nanomaterials, such as silica nanoparticles, carbon nanomaterials, they usually have affluent functional groups on the surface, which can be easily conjugated with other ligands.

4.2. Amphiphilic polymer assembly

Inorganic nanoparticles with high quality are usually prepared in organic phase with hydrophobic surface protection ligands because it allows higher reaction temperature. In addition to the above-mentioned ligand exchange strategy, amphiphilic polymer coating is another widely used method for hydrophobic nanoparticle surface engineering. The polymers encapsulate nanoparticles via hydrophobic interactions between hydrophobic portion of the polymer chains and initial hydrophobic surface coating of nanoparticles. Hydrophilic portion of the polymer chains faces the aqueous media, and renders the nanoparticles soluble in aqueous media. This approach keeps the initial surface coating of nanoparticles and can be more effective in preserving the physicochemical properties of as-synthesized nanoparticles. The representative amphiphilic polymers used for hydrophobic inorganic nanoparticle surface engineering are summarized in Table 3. Nie et al. used a high-molecular-weight (100 kDa) copolymer with an elaborate ABC triblock structure and a grafted 8-carbon (C-8) alkyl side chain to encapsulate hydrophobic ligand, tri-n-octylphosphine oxide (TOPO) protected CdSe/ZnS QDs.[74] A key finding is that this polymer can disperse and encapsulate single TOPO-capped QDs via a spontaneous self-assembly process. As a result, after linking to PEG molecules, the polymer-coated QDs are perfectly protected, so their optical properties did not change in a broad range of pH (1 to 14) and salt conditions (0.01 to 1 M). Dai et al. reported the synthesis of several poly(ethylene glycol) grafted branched polymers based on poly(γ-glutamic acid) and poly(maleic anhydride-alt-1-octadecene) for functionalization of various nanomaterials including single-walled carbon nanotubes (SWNTs), gold nanoparticles, and gold nanorods, affording high aqueous solubility and stability for these materials.[75] Moreover, the polymer-coated SWNTs exhibited remarkably long blood circulation upon intravenous injection into mice.

4.3. Electrostatic layer-by-layer assembly

Electrostatic layer-by-layer assembly is another easy-handling strategy for inorganic nanoparticle surface engineering because of the strong electrostatic attraction between

oppositely charged species. Li et al. have developed a layer-by-layer (LBL) assembly strategy that uses oppositely-charged linear polyions to generate water-soluble UCNPs.[81] Upon sequential adsorption of positively charged poly(allylamine hydrochloride) (PAH) and negatively charged poly(sodium 4-styrenesulfonate) (PSS) onto the surface of nanoparticles, they successfully modified NaYF4:Yb/Er nanoparticles with stable amino-rich shells. The LBL assembly technique offers many advantages, albeit requiring repeated wash steps after each adsorption step, and these include simplicity, universality, and thickness control in nanoscale. More importantly, the high stability and biocompatibility of these polyions make them attractive as coating materials for a wide range of fundamental and technological applications.

4.4. *In-situ* ligand coating during synthesis

Taking advantage of the easy-preparation nature of some kinds of inorganic nanoparticles, such as AuNPs, AgNPs, IONPs, the organic-inorganic hybrid nanoparticles could also be prepared in one-pot reaction containing the inorganic precursors and organic ligands. He et al. reported the synthesis of hyperbranched polyethyleneimine-protected silver nanoclusters (hPEI-AgNCs) using a facile, one-pot reaction under mild conditions.[82] The hPEI-AgNCs were very stable against extreme pH, ionic strength, temperature, and photoillumination. IONPs also have been reported to be prepared in the presence of a variety of surface coating ligands, such as dextran, starch, glycosaminoglycan, and polyvinyl alcohol (PVA), to obtain biocompatible and thermodynamically stable dispersions of IONPs in aqueous media. Pierre et al. reported the detailed magnetic and structural properties of IONPs formed in the presence of dextran. The results suggested that the presence of dextran limits the particle size compared to particles prepared without the polymer.[83]

4.5. Host-guest supramolecular ligand self-assembly

Host–guest interaction mediated self-assembly of hydrophilic ligands on the surface of hydrophobic nanoparticles can also be used as an efficient method to make the hydrophobic nanoparticles soluble in water. Li et al. has successfully developed a simple and efficient approach to draw Adamantane (Ad)-coated NaYF4:Yb, Er nanoparticles into water by the interaction between the host molecule β-cyclodextrin (β-CD) and the guest Ad molecules.[84] This method provided a simple post-treatment through only stirring or shaking, rapid response time (<20 s), high conversion yield (>95%) and exhibited good capability for bioimaging. High quality inorganic nanoparticles are usually synthesized in organic phase with hydrophobic surface coating ligands, such as oleylamine, oleic acid, etc. To transfer the hydrophobic ligands coated nanoparticles into water-soluble forms, a host-guest strategy based on the interaction of α-cyclodextrin (α-CD) and hydrophobic carbon chain was developed. Hydrophobic IONPs, AgNPs and UCNPs were all reported to be transferred to aqueous solution through hydrophilic α-CD self-assembly on the nanoparticle surface to afford aqueous suspensions of nanoparticles with very good stability.[85, 86] This approach is considered to be a generic method for the stabilization of hydrophobic ligands capped inorganic nanoparticles in aqueous solutions, which is an important aspect for the exploration of biological applications of inorganic nanoparticles.

4.6. Lipid shell coating

Lipids are widely used as delivery vectors for small molecular drugs and nucleic acid gene drugs of various molecular weights. Lipids are considered to be highly biocompatible and non-immunogenic. Lipid shell coating is a very well-established method for inorganic nanoparticle surface engineering to produce hybrid nanosystem with high biocompatibility. Huang et al. reported a lipid-coated calcium phosphate (LCP) nanoparticle formulation for efficient delivery of siRNA to a xenograft tumor model by intravenous administration.[87] After entering the cells, LCP would decompose at low pH in the endosome, which would cause endosome swelling and bursting to release the entrapped siRNA. Inorganic nanoparticles with hydrophobic surface ligands (such as QDs, IONPs) also can be hydrophilized by coating with lipid shell for biomedical applications.[88, 89] Anderson et al. developed a simple and efficient method to coat IONPs with lipid molecules with low polydispersity.[89] Cationic lipids-coated hybrid IONPs showed potent capability to deliver DNA and siRNA into cells. Moreover, the application of an external magnetic field further enhanced the efficiency of nucleic acid delivery.

5. Metallic Core Hybrid with Organic Shell Delivery System

There has been a long history for metals to be used in disease treatments since ancient times. Copper and iron were recorded for reducing inflammation and treating anemia, respectively, in ancient compendiums.[90] More modern written accounts published at the beginning of the 20th century reported the use of sodium vanadate to lower blood sugar levels in diabetic patients and simple gold complex to treat rheumatoid arthritis.[91] The greatly successful development of platinum-based anticancer drugs attracted tremendous attention for medicinal inorganic chemistry.[90] Along with the advent of nanotechnology era, many kinds of metal-based nanoparticles have been prepared and great efforts have been paid to explore their biomedical applications. These metal-based nanoparticles, including Gold Nanoparticles (AuNPs), semiconductor quantum dots (QDs), iron oxide nanoparticles (IONPs), up-conversion nanoparticles (UCNPs), and so on, have been extensively developed for detection, imaging, delivery and therapy applications. Owing to their unique optical, magnetic, electronic and thermal properties, versatile functions can be endowed to the engineered delivery systems. For example, the real-time delivery process can be tracked by optical imaging or magnetic resonance imaging (MRI), smart cargo release can be realized through pH or thermal responsive design, and much more potent therapy efficiency can be achieved by combining the intrinsic therapeutic effects of the metal nanoparticles. Moreover, the composition, size, shape and surface properties of these nanoparticles can be flexibly and precisely tailored, which afford great superiority for different biomedical applications.[92] In this section, we will introduce representative classes of metal-based nanoparticles engineered as inorganic-organic hybrid delivery systems of siRNA therapeutics.

5.1. Gold Nanoparticles

Gold is known to be one of the least reactive metals, exhibiting incredible chemical resistance against both oxidation and corrosion. The biocompatibility of Au nanomaterials has been widely demonstrated by a number of *in vitro* and *in vivo* studies.[93–95] Meanwhile, Au nanostructures can be readily synthesized with great diversity in size and shape, which is

considered to be a crucial factor to determine the fate of nanocarriers, such as the blood circulation time, organ specific accumulation and cellular uptake mechanism. The size of synthetic AuNPs usually ranges from several nanometers to several hundred nanometers. Most commonly investigated morphologies include gold nanosphere, gold nanorod, gold nanoshell, gold nanocage, gold nanocluster, and so on (Fig. 6A). Moreover, gold nanostructures possess some other unique properties for biomedical applications, such as strong localized surface plasmon resonance (LSPR), X-radiation absorption ability, photo-thermal conversion properties, and so on.[96] Therefore, when nanocarrier strategy emerged as a promising method to address the barriers of naked siRNA administration, gold nanoparticles immediately caught the attention of researchers as an excellent candidate to achieve this goal.[97, 98] In the past decade, various kinds of AuNPs-based delivery systems have been developed through well-defined organic surface engineering with biocompatible polymers or biomolecules for siRNA delivery.

As introduced above, a well-characterized approach for surface engineering of gold nanostructures is utilizing their ability to form very strong and stable gold-thiolate bonds (Au-S) with molecules containing thiol (-SH) or disulfide groups (S-S) (Fig. 6B).[93] Park et al.[99] synthesized AuNPs in the presence of cysteamine hydrochloride to afford amine-functionalized AuNPs with positively charged surface. Then siRNA conjugated with PEG_{5000} with a disulfide linker was added to adhere to the surface of AuNPs through electrostatic interaction. The nanosized polyelectrolyte complexes could be efficiently internalized into human prostate carcinoma cells and release siRNA in reductive cytosol environment. Thus, enhanced intracellular uptake of siRNA and significant inhibition of the target gene expression were achieved. Anderson et al.[100] employed a different strategy for siRNA-PEG co-loading with AuNPs by first decorating the 15 nm AuNPs with SH-PEG_{1000}-NH_2 and then conjugating siRNA by means of a disulfide crosslinker to the terminal of the PEG. A comparable loading of ~30 strands of siRNA per nanoparticle has been reported. To enhance the cellular uptake and facilitate endosomal escape, these particles were further coated with different kinds of poly(β-amino ester)s (PBAEs), a new class of cationic biodegradable polymer. These nanoparticulate formulations finally realized high levels of *in vitro* siRNA delivery, achieving gene silencing as good or better than the commercially available lipid reagent, Lipofectamine 2000. Other authors have taken another way to address the endosomal escape issue and realize laser-triggered controlled release of siRNA by introducing gold nanoshells as the core structure of the delivery system. Gold nanoshells with peak plasmon resonance in the near-infrared (NIR) range are susceptible to local heating under irradiation of NIR light (Fig. 6C).[101, 102] A poly-$_L$-lysine peptide (cysteine-tyrosine-serine-$lysine_{50}$) was used to modify gold nanoshell surface, affording a positive charge of 14.27 mV to capture siRNA electrostatically. Controlled release of siRNA and enhanced endosome rupture have been achieved by irradiating with lower power NIR irradiation without causing obvious cytotoxicity.[102]

Gold nanoparticles have also been developed for co-delivery of siRNA and small molecular chemotherapeutic drugs through well-defined surface organic ligand engineering.[103, 104] Gong et al. reported the surface engineering of AuNRs for co-delivery of achaete-scute complex-like 1 (ASCL1) siRNA and doxorubicin (Dox).[103] AuNRs were covalently modified with different functional components: a pH-labile hydrazone linkage tethering Dox

to enable pH-controlled drug release, a polyarginine cationic polymer for complexing siRNA and a tumor-targeting ligand octreotide (OCT), to specifically target neuroendocrine cancer cells. Finally, the delivery system Au-DOX-OCT-siRNA resulted in significantly higher cellular uptake and gene silencing effect. The OCT targeting nanocarrier-mediated combination chemotherapy and RNA silencing exhibited the strongest anti-proliferative effect.

In addition to covalent modification of gold nanostructures with thiol-containing ligands, layer-by-layer assembly is an alternative way to engineer multifunctional surface for siRNA delivery.[105–111] The commonly used AuNPs are synthesized in aqueous solution by a citrate reduction method, resulting in a negatively charged surface. Poly(ethylene imine) (PEI), a widely used positively charged polymer in gene delivery study, has been employed to conduct the layer-by-layer assembling process.[105] In this work, two kinds of final delivery systems with different terminal surfaces were fabricated, one with a terminal siRNA layer while another one with a terminal PEI layer. The final size of these particles is reported to be within 20-25 nm range, with a loading density of about 780 siRNA per particle. The cellular particle uptake of siRNA/PEI-AuNPs was significantly more than PEI/siRNA/PEI-AuNPs; however, transmission electron microscopy (TEM) images showed siRNA/PEI-AuNPs particles primarily trapped within the endosome. Finally, GFP silencing studies showed that PEI/siRNA/PEI-AuNPs achieved 70% GFP knockdown 48 h post transfection while no knockdown for particles with siRNA/PEI-AuNPs, suggesting a need of endosome escape component of siRNA delivery vectors. Liang et al. reported the incorporation of an anionic charge-reversal polyelectrolyte (PAH-Cit, cis-aconitic anhydride-functionalized poly(allylamine)) to assemble with PEI and siRNA on AuNPs surface through layer-by-layer method (Fig. 6D).[106, 107] The PAH-Cit can undergo a charge reversal from negative to positive inside the lysosome during a pH change from 7.4 to 5.0, which will disassemble the PEI/siRNA/PAH-Cit/PEI-AuNPs layer-by-layer complex to accelerate siRNA release. The silencing efficiency of targeting gene was reported to be 80%, compared to only 20% for complexes formed with noncharge-reversal polymer. Confocal images revealed that enhanced gene silencing effect benefited from the increased endosomal escape ability of this charge-reversal complex. This layer-by-layer strategy was also used to fabricate PEI modified gold nanorods (AuNRs) for siRNA delivery with combination of photothermal therapy.[109–111] Cancer is a complicated disease that usually requires combination therapy of several treatment modalities. Photothermal therapy is an effective and noninvasive cancer treatment procedure, which generally utilizes a NIR light source to activate tumor localized photothermal agents to trigger local hyperthermia. AuNRs exhibit strong photo-thermal conversion effect under NIR light irradiation because of the surface plasmon field enhancement of the absorption. We demonstrated that PEI-Au NRs not only protect siRNA from degradation, but also facilitate endosomal escape, both of which are prerequisites for successful gene silencing.[109] As a result, the combined anticancer activity of PEI-Au NR/siRNA, PKM2 gene inhibition and photothermal effect showed very potent anticancer efficiency against breast cancer cells.

Moreover, taking advantage of their ease of synthesis property, gold nanoparticles surface engineering with positive organic ligands for siRNA delivery could be readily realized *in-situ* during the process of particle synthesis.[112–114] Wang et al. reported the manufacturing

of PEI-capped AuNPs by directly using PEI_{25k} as the reductant and stabilizer, in place of citrate.[112] This resulted in AuNPs with a positive surface charge which loaded siRNA molecules via electrostatic interactions. It was reported that PEI-AuNPs induce more significant and enhanced reduction in targeted green fluorescent protein expression in MDA-MB-435s cells, because of more siRNA internalization, as evidenced by confocal laser scanning microscopy observation and fluorescence-activated cell sorting analyses. Furthermore, lower cytotoxicity of PEI-AuNPs than pure PEI was observed at siRNA concentration of 120 nM. Another study took a similar *in-situ* strategy and synthesized AuNPs in the presence of a block polymer p($HPMA_{70}$-b-$DMAPMA_{24}$).[113] This resulted in AuNPs with diameters about 7 nm surrounded by a HPMA block, which serves as a hydrophilic, sterically stabilizing shell (similar to the role of PEG). The DMAPMA block functionalized as the cationic part for binding siRNA. The gene silencing efficiency on luciferase-expressing KB cells were evaluated to up to 50% under the condition of 100 nM siRNA, 6 h transfection and 24 h incubation. In recent years, fluorescent gold nanoclusters have attracted a lot of attention due to their unique features, such as ultrasmall sub-nanometer size, great biocompatibility and excellent photostability, making them ideal fluorescent labels for biological applications.[115, 116] In a recent study, positively charged gold nanoclusters (GNC) were synthesized through on-step reduction of Au^{3+} in the presence of thiolate-containing GSH and oligoarginine CRRRRRRRRR.[114] The prepared GNCs had a well-defined core structure with diameters around 2.6 nm. It was reported that the GNCs had a loading capacity of 226 μmol siRNA per gram GNCs. Nerve growth factor (NGF) siRNA was loaded for pancreatic cancer treatment. The GNC-siRNA complex exhibited several attractive properties, increasing the serum stability of siRNAs, prolonging the blood circulation lifetime of siRNA and enhancing the cellular uptake and tumor accumulation of siRNA. Subsequently, GNC-siRNA complexes effectively down-regulated the NGF expression in both Panc-1 cells and pancreatic tumor model. Finally, effective inhibition of the tumor progression was achieved in three pancreatic tumor models (subcutaneous model, orthotopic model and patient-derived xenograft model) without adverse effects.

5.2. Quantum Dots

In the past two decades, quantum dots (QDs), also known as semiconductor nanoparticles, have become one of the dominant classes of fluorescent dyes in various biomedical areas since the pioneered works at 1998.[117–121] QDs are generally made from hundreds to thousands of atoms of group II and VI elements (e.g. CdSe and CdTe) or group III and V elements (e.g. InP and InAs) with diameters on the order of 2-10 nm.[122] QDs exhibit unique optical and electronic properties, such as size-tunable emission, resistance to photobleaching, superior signal brightness, and broad absorption spectra of excitation (Fig. 7A).[121] QDs also have a versatile surface chemistry, thereby allowing further surface engineering of functional groups to facilitate drug loading and cellular uptake. Therefore, QDs have been considered to be promising nanoscale scaffold for designing multifunctional nanosystems with both imaging and therapeutic functions. Though the direct use of QDs as drug delivery vectors remains questionable due to their potential long-term toxicity, QDs offer great potential as a proof-of-concept model to investigate the fate of nanoparticle-based drug delivery systems (NDDS) in biological systems, especially *in vivo*.[121, 123] Owing to

these unique features of QDs, they also have been considered to be a promising candidate for siRNA delivery system to evaluate cellular uptake mechanism,[124] siRNA release process tracking,[125–128] cancer cell targeting,[129, 130] endosome escape,[131] *in vivo* distribution,[132, 133] and so on.

QDs were for the first time introduced into siRNA delivery system by Bhatia et al. for monitoring the delivery process and improving gene silencing effect.[134] In this study, QDs were first modified with mercaptoacetic acid and thiol-PEG_{5000}, and then incorporated into cationic liposomes along with siRNA. Take advantage of the superior photostability and tunable optical properties of QDs, they used flow cytometer to track delivery of nucleic acids, sort cells by degree of transfection and purify homogenously-silenced subpopulations. Moreover, a multiplexed gene knockdown study was achieved using two kinds of QDs different colors for different targeting siRNA labeling. For cellular siRNA delivery, endosome escape is a critical issue, because delivery complexes are usually internalized by cells though endocytosis pathway, easily resulting in enzymatic degradation of the payload siRNA.[124, 131] Gao et al. reported the engineering of QDs surface by coating with proton-absorbing polymer, with a balanced composition of tertiary amine and carboxylic acid groups (Fig. 7B).[124, 131] The intracellular behavior of QD-siRNA complexes, including uptake, transport, and localization in live cells were investigated by fluorescence tracking. It was demonstrated that the proton-sponge coating on the QD surface leaded to efficient siRNA release from intracellular vesicles, which achieved 10-20-fold improvement in gene silencing efficiency.

Cancer cell targeted siRNA vector further improves the cancer specificity and therapeutic efficiency, and active tumor targeting moieties are usually conjugated to the carriers for molecular recognition of unique cancer-specific markers. With excellent fluorescent properties, siRNA nanocarriers engineered from QDs offer great superiority for targeted siRNA delivery tracking, mechanism study and therapeutic efficiency evaluation.[129, 130, 135, 136] Lee et al. developed a multifunctional delivery platform based on QDs for targeted EGFRvIII gene down-regulation (Fig. 7C).[129] RGD and HIV-TAT peptides were conjugated to QDs surface to target the integrin receptor protein $\alpha v\beta 3$ overexpressed on U87 cells and to enhance the cellular transfection efficiency of QDs-siRNA complex. Target-specific delivery of siRNA was demonstrated by tracking the QDs fluorescence in a novel co-culture system containing the U87-EGFP cell line with other less-tumorigenic cell lines, such as PC-12 cells. In another study, chimeras composed of aptamer targeting prostate-specific membrane antigen (PSMA) and siRNA were delivered by positively charged QDs which were engineered from coating hydrophobic QDs with amphiphilic copolymers poly(maleic anhydride-alt-1-tetradecene) and then conjugating with PEI.[130] The central concern of this work is to evaluate the cellular uptake efficiency of chimeras-QDs complex established through two different approaches: one-step direct adsorption chimaeras on QDs and two-step method by sequential adsorption siRNA and conjugation aptamer to siRNA. Finally, fluorescence tracking of QDs demonstrated that the two-step approach could greatly retain the conformation and high accessibility of the targeting aptamer moieties to achieve significantly higher transfection efficiency and targeting gene down-regulation ability.

With well-defined surface organic ligand engineering, QDs could also be used as siRNA and small molecule co-delivery vectors. A series of biocompatible QDs modified with amino acid grafted β-cyclodextrin (β-CD) derivatives have been reported.[137–139] The arginine-β-CD modified QDs (Arg-CD-QDs) not only have positively charged surface to absorb siRNA, but also a hydrophobic cavity of β-CD, which serves as molecular capsule to encapsulate doxorubicin (Dox) (Fig. 7D).[139] The siRNA was designed to target and silence the multidrug resistance gene (MDR-1), which is responsible for multidrug resistance in cancer cells. By simultaneous transportation of siRNA and Dox across the cell membrane to down-regulate the expression of P-Glycoprotein (P-gp), the multidrug resistance could be reversed and as a result the efficiency of Dox can be improved in multidrug resistant cancer cells.

5.3. Iron Oxide Nanoparticles

Magnetic iron oxide nanoparticles (mostly maghemite, γ-Fe_2O_3 or magnetite, Fe_3O_4) are well-established nanomaterials that possess unique magnetic properties. IONPs are being actively investigated as new generation of magnetic resonance imaging (MRI) contrast agents (Fig. 8A & B).[56, 140–143] Owing to their unique characteristics, including efficient contrast effects, excellent biocompatibility and versatile surface functionalization capability, IONPs are extensively explored for various biomedical applications, such as medical diagnosis, detoxification of biological fluids, protein purification, cell separation, chronic iron deficiency anemia treatment, hyperthermia, drug/gene delivery, and so on.[144–146] The excellent biocompatibility of IONPs has been widely demonstrated in a number of studies *in vitro* and *in vivo*, because iron is one of the most abundant endogenous metallic elements in living organisms and is essential for various biological processes. IONPs can be degraded in the body and subsequently incorporated into iron pools or used in metabolic processes.[147] Several IONPs-based nanoformulations have been approved or in the clinical trial as MRI contrast agents or therapeutic agents.[148–150]

MRI has a number of unique advantages including deep tissue penetration, high spatial resolution, and excellent soft tissue contrast.[140] Therefore, IONPs-based delivery systems possess great promise for simultaneously *in vivo* transportation tracking, biodistribution imaging, and drug accumulation evaluation. Moore et al. developed a dual-purpose probe by surface engineering of IONPs for *in vivo* transfer of siRNA and the simultaneous imaging of its accumulation in tumors by high-resolution MRI and near-infrared *in vivo* optical imaging (Fig. 8C).[38] The multifunctional delivery vector is synthesized through a step-by-step method IONPs was first coated with dextran and then conjugated with Cy5.5 dyes and covalently linked to siRNA molecules specific for model (GFP) or therapeutic (survivin) targets, and this nanocarrier is also modified with myristoylated polyarginine peptides (MPAP) serving as a membrane translocation module. The *in vivo* transportation of siRNA mediated by the IONPs could be monitored by MRI and optical imaging. In addition, the imaging results of gene silencing process could correlate with histological data. In another study, a similar all-in-one strategy is used for siRNA delivery and simultaneously *in vivo* transportation monitoring. Bovine serum albumin (BSA)-coated manganese-doped magnetism-engineered iron oxide (MnMEIO) nanoparticles were used as the core material.[151] The MnMEIO NPs were further modified with Cy5 dyes for subcellular imaging and cyclic RGD peptides for cancer cell targeted delivery. The multifunctional theranostic

nanovector was designed to enable highly accurate imaging to be carried out simultaneously with the delivery of therapeutic siRNA, which can minimize invasiveness and deleterious side effects.

To engineer IONPs for specific biomedical applications, surface functionalization with proper organic ligands should be first carried out to make them water-soluble, biocompatitable, or to introduce functional groups for further conjugation. The strategies for IONPs surface engineering for siRNA delivery could be divided into several categories: amphiphilic polymer coating,[152–154] ligand exchange[155–157] and *in-situ* coating during the synthesis process.[158] Chen et al. used amphiphilic Alkyl-PEI2k as surface engineering ligand to assemble with hydrophobic IONPs to form cationic clusters for binding siRNA (Fig. 8D).[159] The cluster nanocarrier could protect siRNA from enzymatic degradation in serum, and release complexed siRNA efficiently in the presence of polyanionic heparin. The excellent gene silencing efficiency of the siRNA-loaded system is assessed with 4T1 cells stably expressing luciferase (fluc-4T1) and with a fluc-4T1 xenograft model. Moreover, unlike high-molecular-weight analogues, the Alkyl-PEI2k-coated IONPs show good biocompatibility. Pierre et al. synthesized two types of polymers, i. e. homopolymers poly(oligoethylene glycol) methyl ether acrylate (p(OEG-A)), poly(dimethylaminoethyl acrylate) (p(DMAEA)) and block poly(DMAEA-b-OEG-A) polymers, as the surface exchange ligands.[160] IONPs with a diameter of about 8 nm were obtained. These polymers were grafted separately to IONPs using the strong affinity of phosphonic acid terminal group to IONPs surface yielding IONPs@p(OEG-A), IONPs@p(DMAEA) and IONPs@p[(DMAEA)-b-(OEG-A)]. These polymers confer a good stability to IONPs in aqueous solution and 50% fetal bovine serum (FBS)medium because P(OEG-A) chains effectively mask the positive charge originating from p(DMAEA), thereby limiting protein adsorption on these particles. Hybrid nanoparticles were exploited for siRNA complexation, thereby generating IONPs/siRNA nano-carriers with anti-fouling p(OEG-A) shells. The excellent gene silencing efficiency was achieved on human neuroblastoma SHEP cells both in the presence and in the absence of a magnetic field in FBS containing medium. Take advantage of the well-established silica coating chemistry, a layer of silica shell could be first deposited on the IONPs surface as a platform for further modifications to generate multifunctional delivery system. Magnetic mesoporous silica nanoparticles (M-MSNs) were constructed to load siRNAs into the mesopores of M-MSNs, followed by polyethylenimine (PEI) capping, PEGylation and fusogenic peptide KALA modification.[161] The resultant delivery system exhibited prolonged half-life in bloodstream, enhanced cell membrane translocation and endosomal escapablity, and favorable tissue biocompatibility and biosafety. The therapeutic effect of this delivery system carrying vascular endothelial growth factor (VEGF) siRNA was proved on both subdermal and orthotopic lung cancer models with remarkable tumor suppression, while tumor metastasis was also significantly reduced, overall leading to improved survival.

5.4. Upconversion Nanoparticles

Lanthanide-doped upconversion nanoparticles (UCNPs) exhibit unique luminescent property to utilize sequential absorption of multiple photons through the use of long lifetime and real ladder-like energy levels of trivalent lanthanide ions to produce higher energy anti-Stokes

luminescence. It thereby converts two or more long-wavelength excitation photons, generally NIR light, into shorter wavelength emissions (e.g., NIR, visible, and UV) through a process known as photon upconversion (Fig. 9A).[162] UCNPs possess a lot of advantages for bioimaging, such as high tissue penetration depth, low autofluorescence background, sharp emission bandwidths, large anti-Stokes shifts, high resistance to photobleaching, and high temporal resolution.[163–166] In recent years, UCNPs have been extensively developed as a new class of luminescent agents that have become promising candidates for use in various biomedical applications comprised of imaging, drug delivery, and therapy. [167–169]

Owing to the unique and amazing luminescence property of UCNPs, they have been considered to be promising core structure for light-triggered delivery systems. Light-triggered drug delivery platforms have emerged as an elegant and non-invasive system for drug payload release in a remote spatiotemporal controllable manner at the desired site and time. This control is considered crucial to boost controlled local effective drug accumulation while minimizing side effects, therefore resulting in improved therapeutic efficacy.[170–173] UCNPs offer an excellent choice for this task due to their utilization of NIR-excitation wavelength exhibiting ability to penetrate deeply into living tissues without causing phototoxic effects, which shows great superiority over high energy UV/visible light and expensive high intensity pulsed laser to activate the photosensitive component. In recent years, great breakthroughs have been successfully achieved by utilizing UCNPs system to realize NIR light-trigged drug release for chemotherapy. This concept has also been successfully applied for siRNA delivery and NIR light-triggered activation.[174–178] Zhang et al. reported the exploitation of the potential of NIR-to-UV UCNPs to achive photoactivation of caged siRNA for photo-controlled gene regulation (Fig. 9B).[175] In this study, siRNAs were caged with light-sensitive 4,5-dimethoxy-2-nitroacetophenone (DMNPE) and loaded into the pores of mesoporous silica coated UCNPs by physical adsorption. DMNPE-caged siRNA molecules were shown to be uncaged and turned to functional gene as designed to silence the targeting gene after activated with NIR light irradiation. This concept was fully proved by the significant decrease in GFP fluorescence for caged GFP siRNA delivery in GFP expressing B16 cells and further more *in vivo* in animal models with deep tissue penetration. In another study, Xing et al. developed UCNPs with surface functionalization by cationic photocaged linkers through covalent bonding, which could effectively adsorb anionic siRNA through electrostatic attractions (Fig. 9C).[176] The UCNPs-siRNA complexes could be easily internalized by living cells. Upon NIR light irradiation, the photocaged linker on the UCNPs surface could be cleaved by the upconverted UV light and thus initiated the intracellular release of the siRNA. The *in vitro* agarose gel electrophoresis and intracellular imaging results indicated that the UCNPs-based gene carrier system allowed effective siRNA delivery. The applications of NIR light instead of direct high energy UV irradiation may greatly guarantee less cell damage.

UCNPs are also shown to be an alternatively new choice for photodynamic therapy (PDT) with NIR light excitation.[179, 180] This promising approach was introduced for efficient siRNA delivery and therapy by combination with photochemical internalization, PDT or photothermal therapy (PTT) to achieve synergistic tumor therapy effect.[181–183] Positively charged UCNPs were engineered via a layer-by-layer strategy and further loaded simultaneously with Chlorin e6 (Ce6), a photosensitizing molecule, and siRNA, which

targets the Plk1 oncogene (Fig. 9D).[182] Under excitation by a NIR light at 980 nm, cytotoxic singlet oxygen can be generated via resonance energy transfer from UCNPs to photosensitizer Ce6, while the residual upconversion luminescence is utilized for imaging. The silencing of Plk1 induced by siRNA delivered with UCNPs could induce significant cancer cell apoptosis. As the result of such combined photodynamic with gene therapy, a remarkably enhanced cancer cell killing effect is realized. Gd-doped UCNPs were synthesized and modified with cationic polymers, which were further conjugated with cypate and loaded with HSP70-siRNA against heat shock protein 70 (HSP70).[183] Cypate, a type of organic carbocyanine fluorophore, displays high molar extinction coefficient for NIR light, thereby exhibiting high photothermal conversion efficiency for cancer photothermal therapy. HSP70 are usually upregulated under heat stress, thus protecting cells from hyperthermic damage. Therefore, this UCNPs-cypate-siRNA system could simultaneously generate photothermal effect to destroy cancer cells and inhibit the expression of HSP70 to achieve synergistically enhanced antitumor therapy effect. Moreover, this multifunctional platform provided a multimodal imaging-guided manner for precisely controllable antitumor theranostics.

6. Non-metallic Core Hybrid with Organic Shell Delivery System

The intrinsic physical and chemical properties of non-metallic materials also play a critical important role in the development progress in medicine and other industries. As well known that the arsenic trioxide, also named *pi-shuang* in traditional Chinese medicine.[184] It has long been of biomedical interest and now used to treat cancer like acute promyelocytic leukemia (APL). Another case, selenium (Se) is an essentials dietary component for humans, recent increasing reports and evidences to show a promising cancer chemopreventive element.[185] With the rapid development of nanotechnology, it has brought great opportunities to use those non-metallic materials as nanocarriers for gene therapies, chemotherapies and other small molecule drugs. Se-based nanoparticles were successfully used to deliver siRNA to inhibit epidermal growth factor receptor signaling, or tumor microenvironment responsive delivery system to enhance the nanotherapeutics.[186, 187] Specifically, both the silicon and carbon elements in group IV, they are most abundant and significant non-metallic substances in human tissues. These two kinds of typical materials have been designed, synthesized and applied in various research fields including nanomedicine. The advantages of these systems including large surface, stability, biocompatibility, facile synthesis, easy surface functionalization, high penetration capacity to biological barriers, ultra-high loading capacity and so on.[188] Silicon based delivery system, such as mesoporous silica nanoparticles (MSNs), nano size silicon particles and nanoporous silicon microparticles (also named multistage vector, MSV). Additionally, carbon based platform including carbon dots, carbon tubes, graphene, fullerene, nanodiamond and so on.[189–191] Both of them have emerged as a booming area for the development of delivery vehicles and for diagnostic agents and anticancer drugs. Following, we expand the siRNA therapeutics based on silica nanoparticles, silicon nano/microparticles and carbon vectors with further fabrication of organic shells for treatment human diseases.

6.1. Silicon Nano/Microparticles

Silicon nanoparticles have been investigated since 1990s. Silicon offers specific physical and chemical properties, size-dependent multicolor reflection of light, photobleaching stability and favorable non-toxicity.[192] Silicon is an “indirect band-gap material” unlike other semiconductors and shows particular changes when its size approximates the bulk Bohr radius (4 nm). Porous silicon with the advantages of biocompatibility, biodegradability and unique physical properties has great potential as a drug delivery platform for various therapeutic agents including chemotherapeutics, gene, and small molecular diagnostics.[193, 194] The *in vivo* biosafety and efficacy are highly dependent on the surface functionalized properties (size, fluorescent dye, peptides, targeting moieties and geometries), administration dosage and routes (Fig. 10A).[195] The typical example of silicon carriers for siRNA delivery is the multistage vector systems (MSVs), this system encompasses three components and each optimized stage to address a different set of biological barriers. 1) Porous silicon microparticle as a first stage component that can be loaded with different nanoparticles. 2) The second stage includes micelles, liposomes, polymeric nanoparticles or metallic nanoparticles, etc. 3) The third stage is the payload drugs including siRNA, chemotherapeutics, miRNA, and antibodies (Fig. 10A).[196]

Up to now, the protocol for mass production of MSV microparticle is easily produced according to the United Stated Food and Drug Administration’s (FDA’s) good manufacturing particles (cGMP). Discoidal MSVs with different diameters (500-2600 nm), various heights (200-700 nm), pore sizes (5-150 nm), and high porosity (40-90%) can be adjusted by modulating the electrochemical etching and photolithography process (Fig. 10B).[197] Consequently, such physical properties of MSVs enable generation of particles with various performance attributes, nanoparticle-loading capacity, drug release profile, biodistribution and degradation kinetics. Additionally, MSVs could successfully stain in the tumor blood vessels because of its geometries properties and the significant difference structure between the tumor and normal vessels. When the silicon microparticles gradually degrade, second stage nanoparticles are released into tumor tissues. These second nanoparticles could further protect the therapeutic drugs from degradation and promote intracellular uptake in cancer cells. Finally, the third stage anticancer drug is typically released from the second stage nanoparticles into targeting cells (Fig. 10C).[195] The MSVs with the novelty of multistage design could sequentially overcome the biological barriers to enhance the accumulation in the disease tissues and improve the drug concentration in the targeting cells.

Based on MSVs system, Ferrari’s groups encapsulated the liposome or polymer siRNA formulated second nanoparticles inside the nanopores successfully applied on treatment of triple negative breast cancer, bone marrow metastatic breast cancer, metastatic ovarian cancer and inflammatory disease.[198–203] Shen *et al.* used the 1000 nm (diameter) and 400 nm (height) MSVs with further optimized through 3-aminopropyl triethoxysilane (APTES) to obtain a slight positive charge, which facilitated loading of slightly negative liposomes into the nanopores. Additionally, APTES fabricated MSVs reveal a more stable ability in the PBS/FBS (10% FBS) mixture solutions. By day 10, most of the porous silicon particles maintain a clear edges and well-defined nanoporous structure. This system could

successfully deliver siRNA inside the SKOV3 (Fig. 10D).[198] Meanwhile, the siRNA with a sustain release from the MSVs to keep the down-regulated protein expression from day 3 to day 9. The *in vivo* results also indicated that this platform could suppress the EphA2 protein expression trigged reduction of total number of microvessels in tumor samples with a dose-dependent behavior. Importantly, tumor growth was completely inhibited when mice treated with MSV/EphA2 siRNA in combination with paclitaxel.

Beside above concept, Shen et al further developed conceptually different from the multistage system. Therapeutic siRNA or microRNA are not pre-packaged, nor are they loaded in the form of nanoparticles in the polycation nanoporous silicon (PCPS) system.[204, 205] Instead, they bind directly to the polycation inside the nanopores, thus providing a very convenient approach to load a large quantity of therapeutic agents. The therapeutic agents and polymer will be slowly released and self-assembled nanoparticles upon silicon particle degradation, thus avoiding a sudden burst of release, their study has clearly demonstrated effective cellular internalization and tumor enrichment (Fig. 10E).[205] Release of therapeutics is achieved during porous silicon degradation, which is another difference from the multistage vector system. This platform with following advantages: 1) with high loading capacity, 2) with low or no toxicity, 3) with a friendly production protocol, and 4) stable during transportation and storage. Additionally, the self-assembled nanoparticles could successfully help siRNA polyplexes to escape from endosome and lysosome to cytoplasm with the benefits from the PEI moieties trigged the proton sponge effects. Furthermore, systemic delivery of PCPS/STAT3 siRNA in murine model of MDA-MB-231 breast cancer enriched particles in tumor tissues and reduced STAT3 expression in cancer cells, causing significant reduction of cancer stem cells in the residual tumor tissue.[205] Totally, their new multistage concept has a high loading capacity and no detectable toxicity. These inorganic (silicon) and organic (liposome or polymer) hybrid systems not only address the biological barriers for siRNA delivery to the targeting tissue and cell, also could maintain a sustain gene silencing expression.

6.2. Mesoporous Silica Nanoparticles

Recently, mesoporous silica nanoparticles (MSNs) have been drawn great attention for nucleic acid transportation due to its biocompatibility, definable morphology, large surface area for massive loading amount of cargos.[206–208] For delivery application, MSNs exhibited unique features on chemical modifications of outer and inner surfaces of MSNs with various bioactive macromolecule including PEG, dendrimers, antibodies, aptamers, peptides and cationic polymer.[208–213] With these various modifications on the surface of MSNs, the siRNA condensed tightly to MSNs by coupling with the cationic polymers and was able to quick uptake due to the target moiety conjugation and following rapid intracellular releasing (Fig. 11A). Therefore, MSNs have also been successfully administered as effective gene delivery devices in different cancers.[214, 215] For instances, Xia et al. studied transfection efficiency of MSNs/siRNA delivery system on GFP-HEPA cells by noncovalent attachment of different molecule weight polyethyleneimine (PEI) polymers to the surface of MSNs as siRNA delivery platform(Fig. 11B).[216] After comparation the cellular uptake, cytotoxicity and transfection efficiency of PEI-coated MSNPs, they demonstrated that 10kDa PEI-coated particles not only bind siRNA with high affinity, but also enable to achieve efficient nontoxic

cellular delivery of these payload. Additionally, due to their unique mesoporous scaffold, MSNs can be easily loaded with both chemotherapy drugs and siRNAs and effectively co-deliver the payloads into tumor cells with synergistic effect.[217–219] Huan et al reported that administration of MSNs loaded concurrently with Doxorubicin and siRNA targeting the P-glycoprotein (P-gp) protein to overcome multiple drug resistance (MDR). They showed that this co-delivery strategy of DOX and siRNA by MSNs was capable to increase the intracellular drug concentration to levels exceeding that of free DOX which resulted in an increased killing of DOX compared with the free form on KB-1V MDR cancerous cell, which demonstrate that it is possible to use the MSNs platform to effectively deliver a siRNA targeted the drug exporter gene that can be used to improve the chemotherapeutic agent sensitivity (Fig. 11 C).[220]

During blood circulating of nanoparticles before reaching their site of action, undesirably releasing and degradation of loaded active siRNA in MSNs may suffered due to the mesoporous structure, which is a major challenge that should be avoided as much as possible.[221] To fulfill this purpose, the porous of MSNs could be capped by materials that only sensitive to the tumor microenvironment, in which that capping molecules respond, following by the opening of MSNP-locked valves to release the siRNA and other active reagent such as chemo drug. Lin et al reported a MSNs based siRNA delivery system with capping the porous by poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) cross-linked with reductive sensitive linker (Fig. 11D).[222] The results showed that the ssCP-MSNs exhibited an excellent siRNA binding capacity. After cellular uptake of ssCP-MSNs, high concentration of intracellular GSH triggers the release of loading siRNA by decapping the PDMAEMA. Moreover, this capping siRNA system exhibited obvious tumor suppression of HeLa-Luc xenograft murine model after following systemic administration. Overall, considering the further evaluation of the anti-cancer activity of MSNs based drug and gene delivery platform, tumor microenvironment-responsive capped MSNs loaded with hydrophobic or hydrophilic chemotherapy drugs in core siRNAs in mesoporous are the most advanced and effective strategy so far.[223, 224] Such responsive capping reagent is decapped because of the specific environment difference of tumor site such as acidic and high redox nature. Administration of such MSNs with the least premature drug and siRNA release behavior significantly will improve the outcome of gene therapy (Fig. 11 E). [225, 226]

6.3. Carbon Vectors

Up to now, carbon-based nanomaterials (CBNs) have been also actively investigated due to their advantageous chemical and physical properties. (i.e., thermal effect, electrical conductivity, and high mechanical strength).[227–229] This perspective highlights different types of carbon-based nanomaterials such as carbon nanotubes (CNTs), graphene, fullerene, carbon quantum dots and carbon fiber currently used in biomedical applications.[230–233] Among the different applications, CBNs are hosting enormous interests as siRNA delivery platform due to their extremely large surface area, with every atom exposed on its surface, which allows for ultra-high functionalization and loading capacities, high affinity with the biology molecule and facile surface functionalization (Fig. 12A).[234–236]

Carbon nanotubes are hollow 1-dimension CBNs with a typical diameter of 1-2 nm and length ranges from 50 nm to 1000 nm. Their tubular morphology endows them to easily and efficiently for cellular uptake by acting as nano-shape needles that make the CNTs become the most widely used CBNs in bio-application .[237–239] Meanwhile, they are capable to encapsulate of cargos and maintain its function with controlled release of loaded molecules which is becoming increasingly important in gene delivery application. For siRNA delivery, both single-walled (SW) and multi-walled (MW) carbon nanotubes were modified or conjugated with siRNA for various disease treatments. Zhang et al. proposed an efficient vector based on SWNTs for siRNA to knockdown murine telomerase reverse transcriptase expression in murine tumor cells on both in vitro and in vivo levels for controlling of key signaling regulators in cancer cells.[240] Guo *et al*, they established the capacity of cationic MWNT to deliver the apoptotic siRNA against PLK1 (siPLK1) in Calu6 tumor xenografts, which express high transfection efficiency comparing with cationic liposome. Moreover, the MWNTs/siRNA complexes significantly improve the xenografts animal survival rate. This study demonstrated the potential therapeutic efficacy of cationic MWNT as a promising safety siRNA delivery (Fig. 12C).[241] Notably, a novel “smart” single-walled carbon nanotubes (SWNTs) to achieve nanotube-siRNA conjugates with reductive cleavable disulfide bond to enable intracellular controlled siRNA releasing from nanotube surfaces was developed by Liu et al as a nonviral molecular transporters for the delivery of siRNA into human T cells and primary cells. This smart siRNA platform exhibited efficient RNA inference of CXCR4 and CD4 receptors on both human T cells and peripheral blood mononuclear cells (PBMCs). The cellular penetrate ability and RNAi efficiency of nanotubes are far exceeding those of existing liposomes due to the underlying hydrophobic interactions on nanotube-mediated molecular delivery (Fig. 12B).[242]

Graphene, a newly discovered two-dimensional carbon nanosheet structure,[243–245] was demonstrated with additional remarkable colloidal stability, easily tunable surface functionalization, and good biocompatibility as a promising nano-carrier for safe and efficient gene transfection.[246–248] Recently, its oxidized form of graphene oxide (GO) were used in a pioneering study by Zhang *et al*. for PEI and PEG dual-modification step as complexing Ckip1-targeted siRNA, following by deposited onto the pre-prepared Titania nanotubes surface via cathodic electrodeposition to obtain the nGO-PEG-PEI/siRNA biofunctionalized implant for enhanced osteogenesis (Fig. 12D). [249] The results demonstrated that together with Ckip-1 siRNA of osteogenic potential, the both osteogenic differentiation on NT-GPP/siCkip-1 implant surface *in vitro* and the new bone formation around implant *in vivo* was significantly promoted which presents a promising implant biomodification siRNA delivery system based on functionalized GO.[249] Ren *et al*, prepared a non-viral carrier (GO-PLL-SDGR) to deliver VEGF-siRNA for targeting cancer therapy. This GO-PLL-SDGR/siRNA complex could deliver VEGF-siRNA into tumor cells and down-regulate the expression of VEGF effectively to inhibit the tumor growth in a S180 xenograft tumor model.[250] These results indicated that targeting cationic GO /siRNA delivery system has great application potential in cancer therapy. Moreover, a GO based siRNA/drug co-delivery system was developed by Zhang *et al*, which concluded a PEI-grafted GO nanocarrier for delivery of Bcl-2 siRNA and doxorubicin. The results reveal that the PEI-GO not only shows significantly lower cytotoxicity but also leads to significantly

enhanced anticancer efficacy that may provide insight into designing and constructing GO based novel drug/gene co-delivery nanocarriers (Fig. 12E).[251]

Other CBNs such as carbon quantum dots (Cdots) are also widely applied on drug/gene delivery due to their photoluminescence natural.[252, 253] For instance, Wu *et al* developed a novel targeting theragnostic nanocarrier based on fc-rPEI-Carbon quantum dots (rPEI-Cdots)/siRNA which can be used simultaneously in lung cancer diagnosis and gene therapeutics. The *in vitro* results showed that treating with fc-rPEI-Cdots/ pooled siRNA complex for 3 days is significantly reduced to nearly 30%.[253] Wang *et al* also demonstrated a Cdots-based and PEI-adsorbed complexes both as imaging agents and Survivin siRNA nanocarriers, which indicate that Cdots-based nanocarriers is a promising platform of both siRNA delivery and imaging for cancer treatment.[254]

Overall, extensive researches have elevated the CBNs as one of the highlighted nanocarrier platform for siRNA delivery owing to their unique combinations of structure, energetic, and electrical properties. The major disadvantage of CBNs to siRNA is their non-biodegradability that may induce a range of adverse health effects. This point has been demonstrated in several studies and need for more future investigations.[255, 256]

7. Conclusion and Perspective

It is encouraging that the recent announcement of Alnylam on the remarkably successful phase III results of Patisiran after two decades of the discovery of RNAi.[257] Meanwhile, resurgence in clinical trials using RNAi occurred in 2012, there are more than 30 RNAi-based therapeutics currently in clinical trials, and several of these are Phase III trials. However, considering the complicated biosystem and time consuming preclinical study, there are still a number of issues to address due to instability of siRNA in body circulation and low bio-application. Luckily, it has been revealed that one of the major challenges indeed for RNAi-based therapy is the development of delivery system. To solve this problem, a large number of carriers have been reported for siRNA delivery including virus and non-virus based platforms during the past two decades.

Among all kinds of siRNA carriers, inorganic-organic hybrid nanoparticles emerge as a diverse set of versatile platforms for both fundamental studies and potential clinical translation applications. (Fig. 13) Here, we summarized the various choices for a broad range of covalent or non-covalent approaches for inorganic nanoparticle surface engineering to provide the ability to further optimize siRNA delivery *in vitro* and *in vivo*. Through the results, it could be concluded that the size and morphology of the inorganic cores showed great flexibility of tailoring to achieve better cellular uptake, blood circulation and specific bio-distribution. Meanwhile, the unique optical, magnetic and thermal properties of inorganic nanoparticles also have been extensively used to explore the crucial mechanisms of siRNA transportation *in vitro* and *in vivo*, such as endosome escape, reticuloendothelial system (RES) capture, kidney clearance, and enhanced penetration and retention (EPR) effect, etc. Decades of studies demonstrated that the therapeutic capability of inorganic-organic hybrid systems for siRNA delivery in a broad range of disease models, showing great promise for future therapeutic applications. However, the remaining obstacles such as

undesired systemic toxic of hybrid systems hinder the translation of these delivery systems into clinic. Therefore, a lot of efforts are still needed to fill the space between basic research and clinical translation for these delivery systems (Fig. 13), for example, engineering and manufacturing siRNA-loaded inorganic nanoparticles with homogeneous size, composition, and surface charge in a simple, fast, and inexpensive manner; revealing the safety effect of siRNA-loaded nanoparticles in regards to administration, clearance route, and potential long-term immune response. Especially, with the development of immunology, more careful evaluation of the immune responses of biological system to the siRNA-nanoparticle complex after administration should be developed. For instance, the potential potently immune system response induced by siRNA molecules and delivery vectors, which is considered to be a great challenge - the contradiction among therapeutic effects from target-specific, RNAi-mediated gene silencing and those caused by nonspecific stimulation (i.e. inflammation/toxicity) of the innate immune system. Better understanding of the fundamental aspects of nanomaterial interactions with biological systems such as organismic, tissue, and cellular levels will further promote the development of this area.

Owing to the great properties of some inorganic nanoparticles, such as the excellent biostability of AuNPs and IONPs, the biodegradability and biocompatibility of silicon materials, great potential for clinical translation applications has enlightened the pathway to the future. Taken together, RNAi therapy based on siRNA has the potential to revolutionize treatment of various kinds of cancers and other diseases. Innovative delivery systems based on inorganic-organic hybrid nanoparticles have great promising to further enrich the fundamental theory of siRNA therapy, and develop safe and efficient delivery platforms for personalized cancer therapy in the future.

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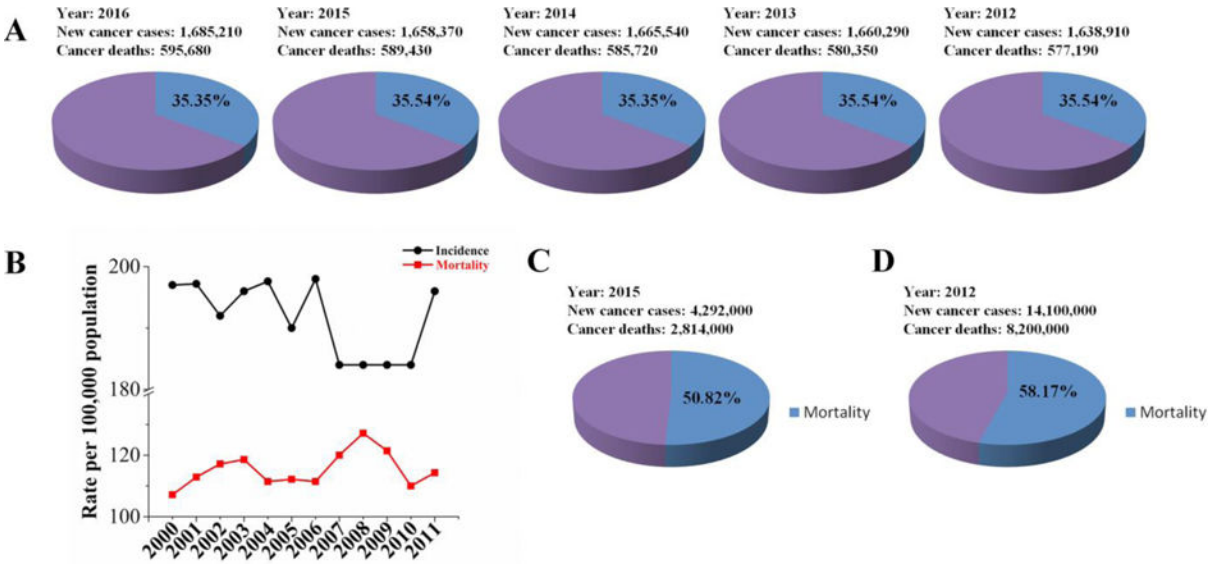

Fig. 1.

Estimated new cancer cases and deaths in United States and China. (A) The new cases and deaths in United States in recent five years. (B) Cancer death statistics in China from 2000 to 2011. (C) The new cases and cancer deaths in China in 2015. (D) The number of new cases and deaths in the world in 2012.

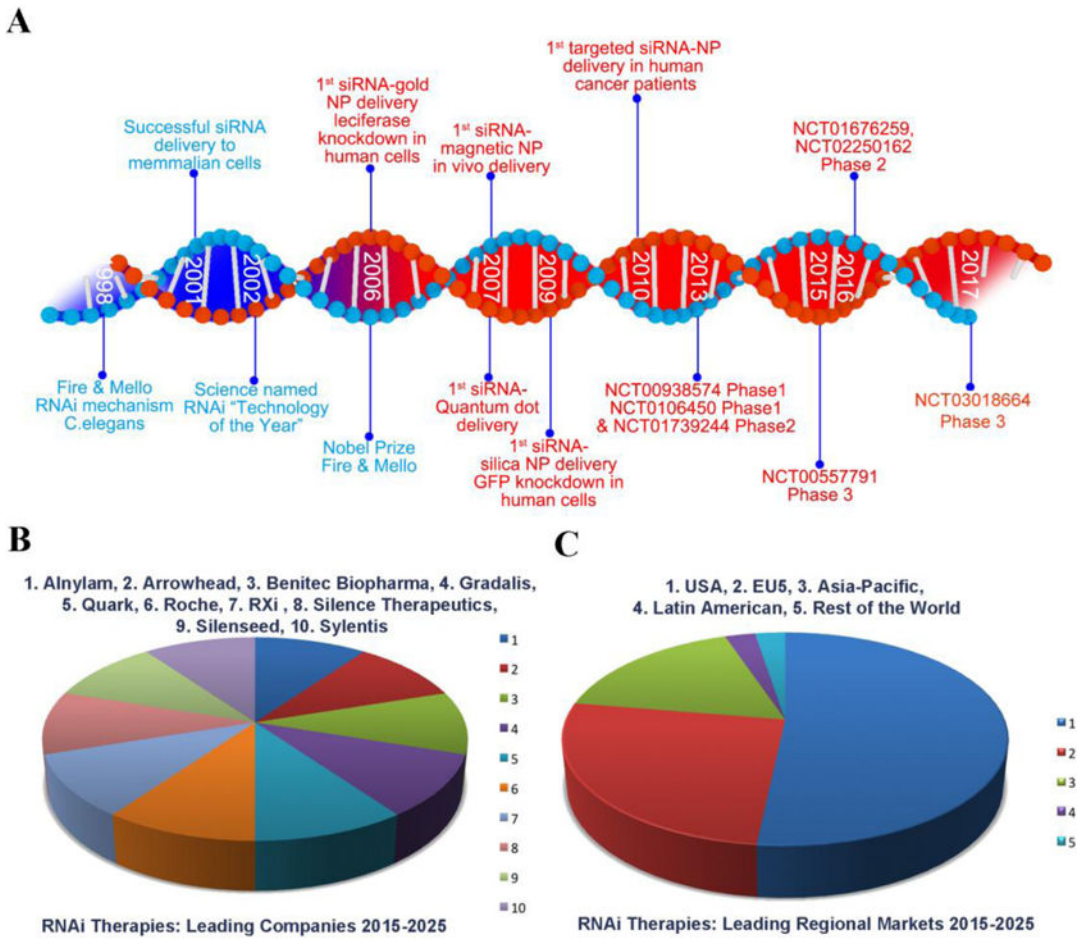

Fig. 2.

Summarization of the greatest events and markets in RNAi therapeutics. (A) Milestone timeline of the last 20 years for the RNAi progress. (B) Leading companies of RNAi therapies in 2015-2025. (C) Leading regional markets of RNAi therapies in 2015–2025.

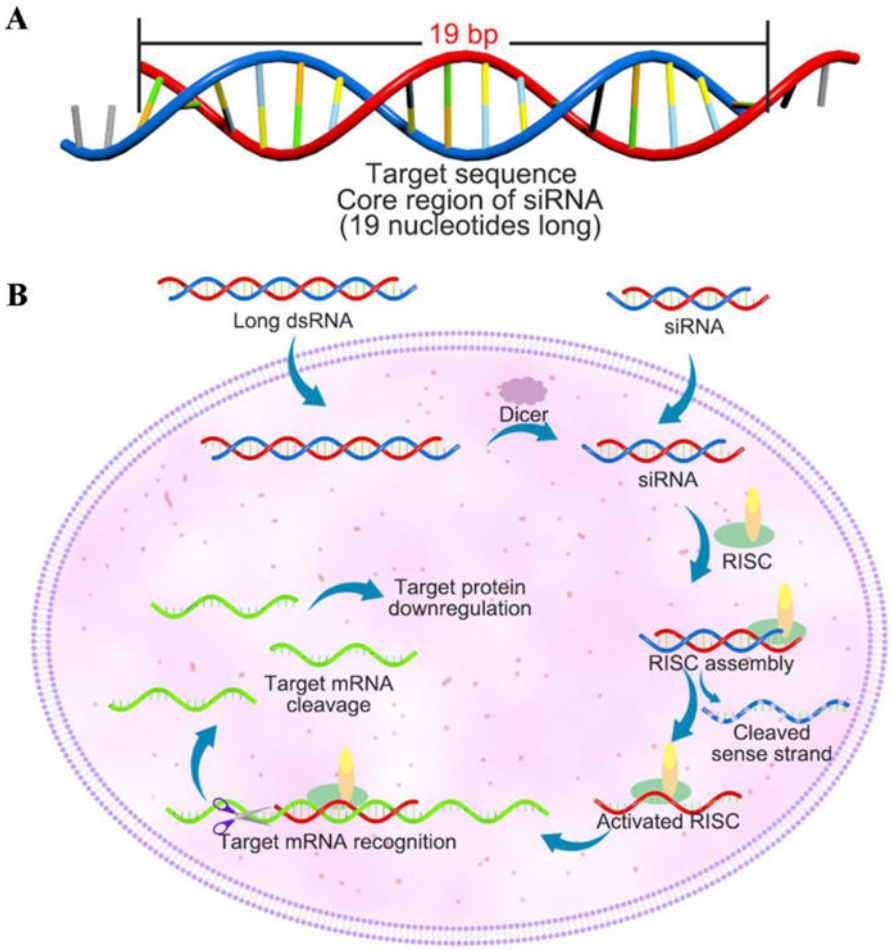

Fig. 3.
Structure and mechanism of siRNA. (A) Structure of siRNA. (B) Action of RNAi.

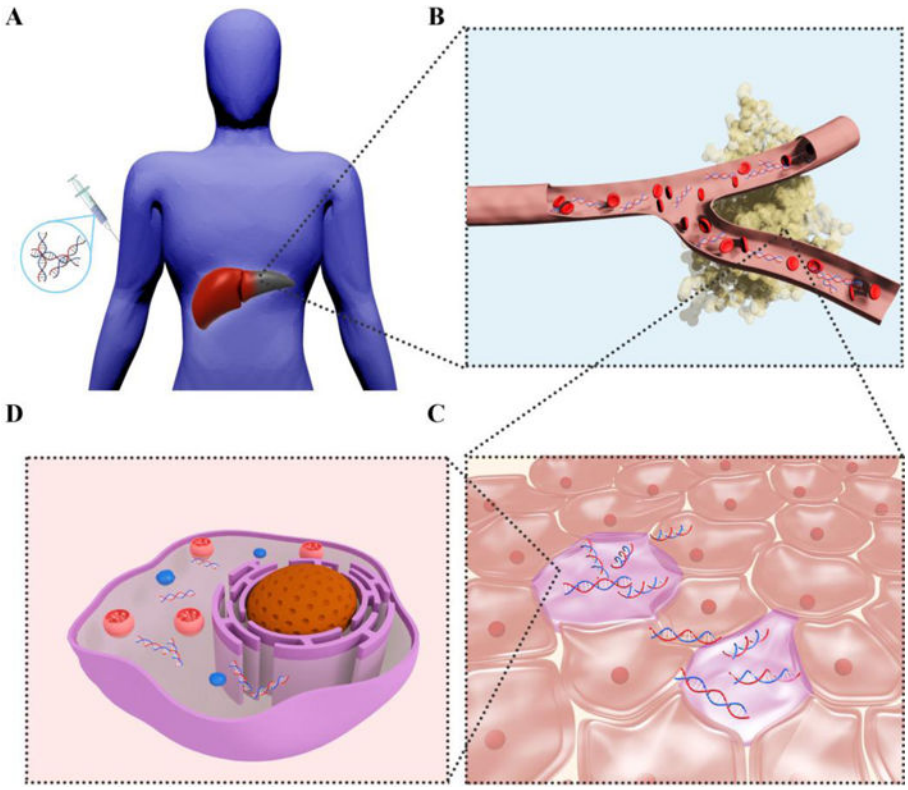

Fig. 4.

Physiological and biological obstacles of siRNA therapeutics. (A) Injection of siRNA therapeutics. (B) Circulation of siRNA in blood vessels. (C) Extracellular behavior of siRNA therapeutics. (D) Intracellular action of siRNA therapeutics.

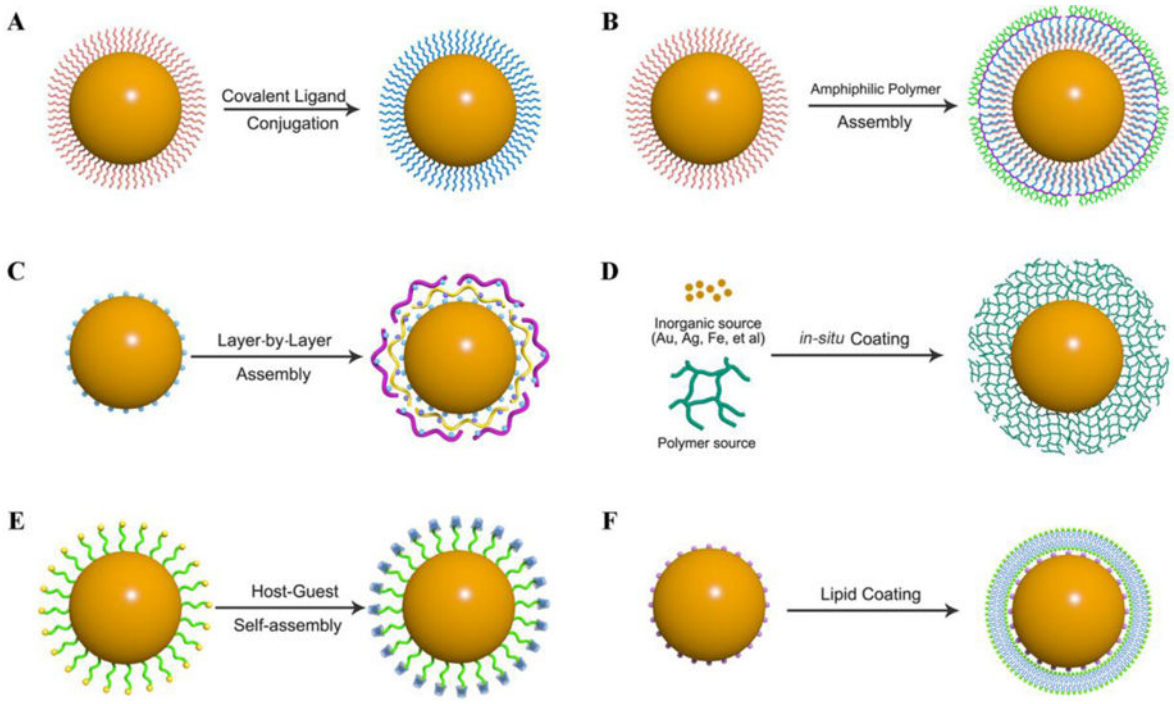

Fig. 5.

The main strategies for inorganic nanoparticle surface engineering. (A) Covalent ligand conjugation. (B) Amphiphilic polymer assembly. (C) Electrostatic layer-by-layer assembly. (D) *In-situ* ligand coating during synthesis. (E) Host-guest supramolecular ligand self-assembly. (F) Lipid shell coating.

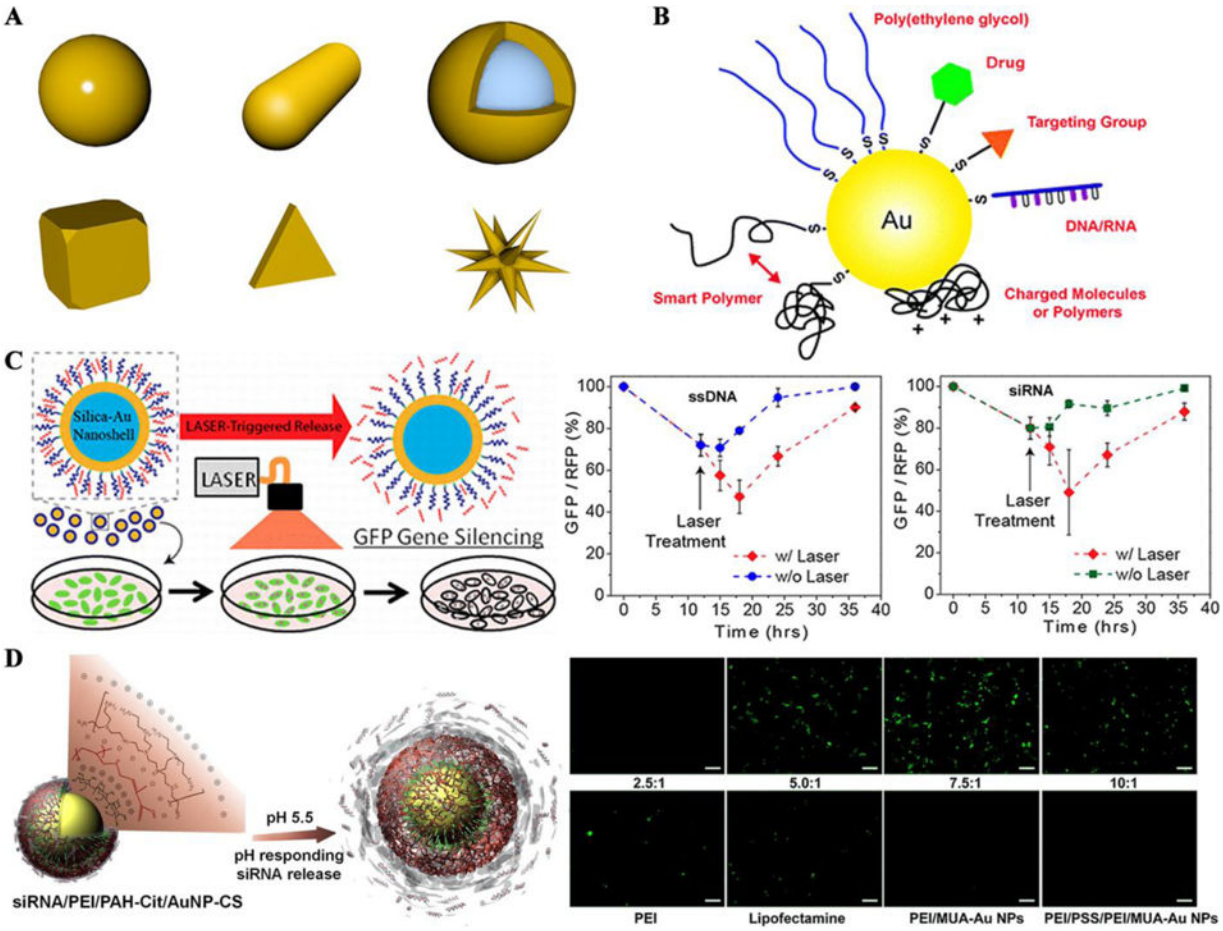

Fig. 6.

Hybrid gold nanoformulated siRNA therapeutics. (A) Illustration of gold nanostructures of different morphologies. (B) Gold nanoparticle surface engineering with a wide variety of functional moieties. (C) Gold nanoshell for siRNA delivery and laser-triggered release. (D) Gold nanoparticles coated with charged reversible polymers via layer-by-layer method for siRNA delivery and pH-responsive release. Figures reproduced with permission from (B) Ref. [93], (C) Ref. [102], and (D) Ref. [106], [107]. Copyright (B) 2011 the Royal Society of Chemistry, (C, D) 2009, 2010 and 2012 American Chemical Society.

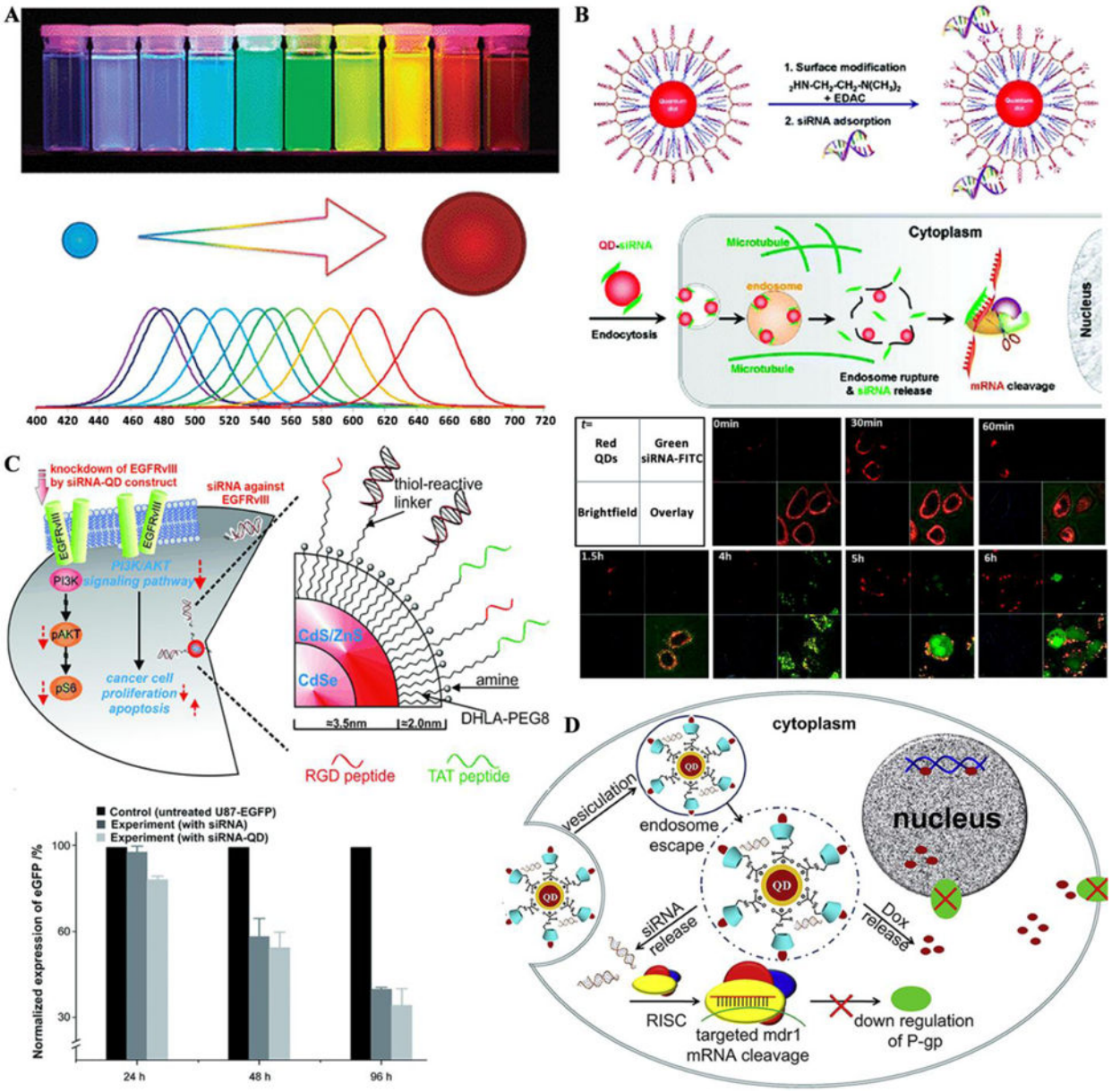

Fig. 7.

Hybrid QDs nanoformulated siRNA therapeutics. (A) The optical property of QDs, size dependent emission wavelength. (B) Proton-sponge coated QDs for siRNA and real-time intracellular siRNA delivery tracking. (C) Multifunctional QDs system for targeted siRNA delivery. (D) L-Argine-β-cyclodextrin modified QDs for siRNA and Dox co-delivery to combat multidrug resistance. Figures reproduced with permission from (A) Ref. [121], (B) Ref. [124], [131], (C) Ref. [129], and (D) Ref. [139]. Copyright (A) 2010 the Royal Society of Chemistry, (B) 2008 American Chemical Society, (C) 2010 John Wiley & Sons, and (D) 2012 Elsevier B.V.

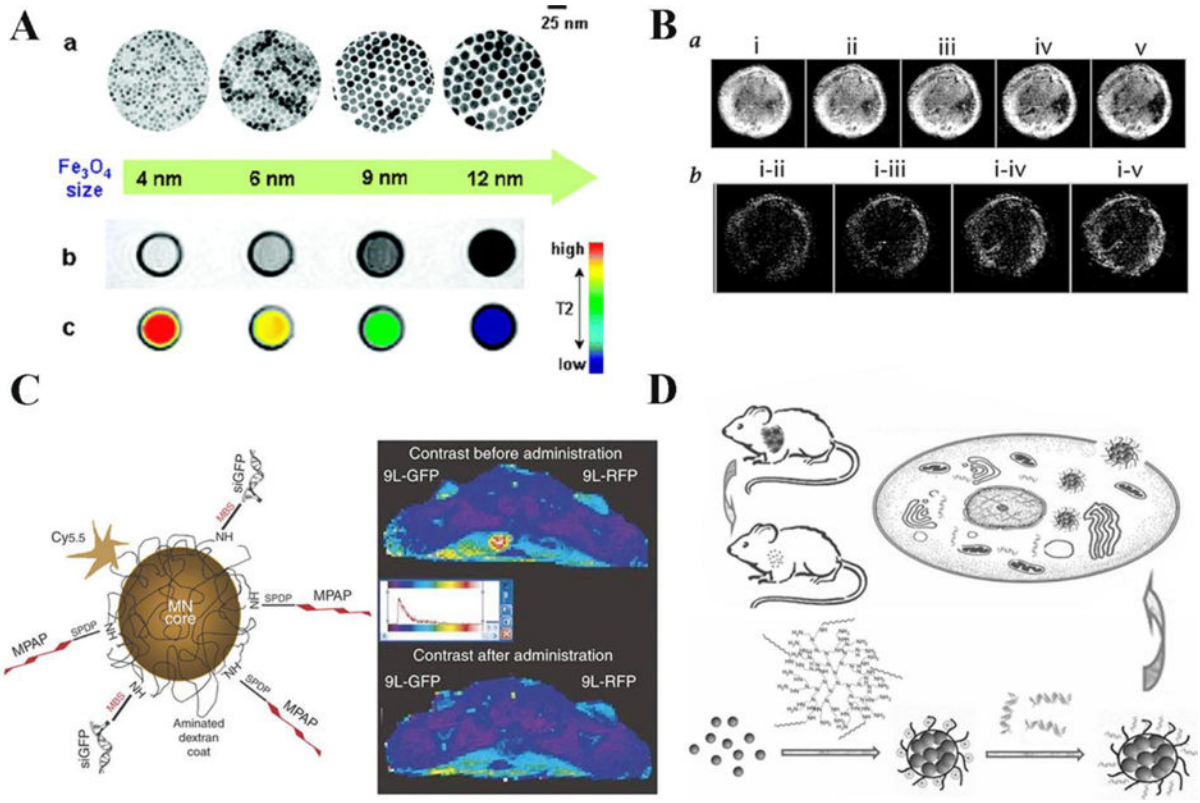

Fig. 8.

Hybrid magnetic nanoformulated siRNA therapeutics. (A) The magnetic resonance imaging property of representative IONPs. Nanoscale size effect on magnetism and induced MR signals. (B) IONPs for targeted *in vivo* tumor imaging. (C) Multifunctional IONPs for *in vivo* siRNA delivery and imaging. (D) Amphiphilic Alkyl-PEI functionalized clustered IONPs for siRNA delivery and imaging. Figures reproduced with permission from (A) Ref. [56], (B) Ref. [142], (C) Ref. [38], and (D) Ref. [159]. Copyright (A) 2005 American Chemical Society, (B) 2001 and (C) 2007 Springer Nature, and (D) 2011 John Wiley & Sons.

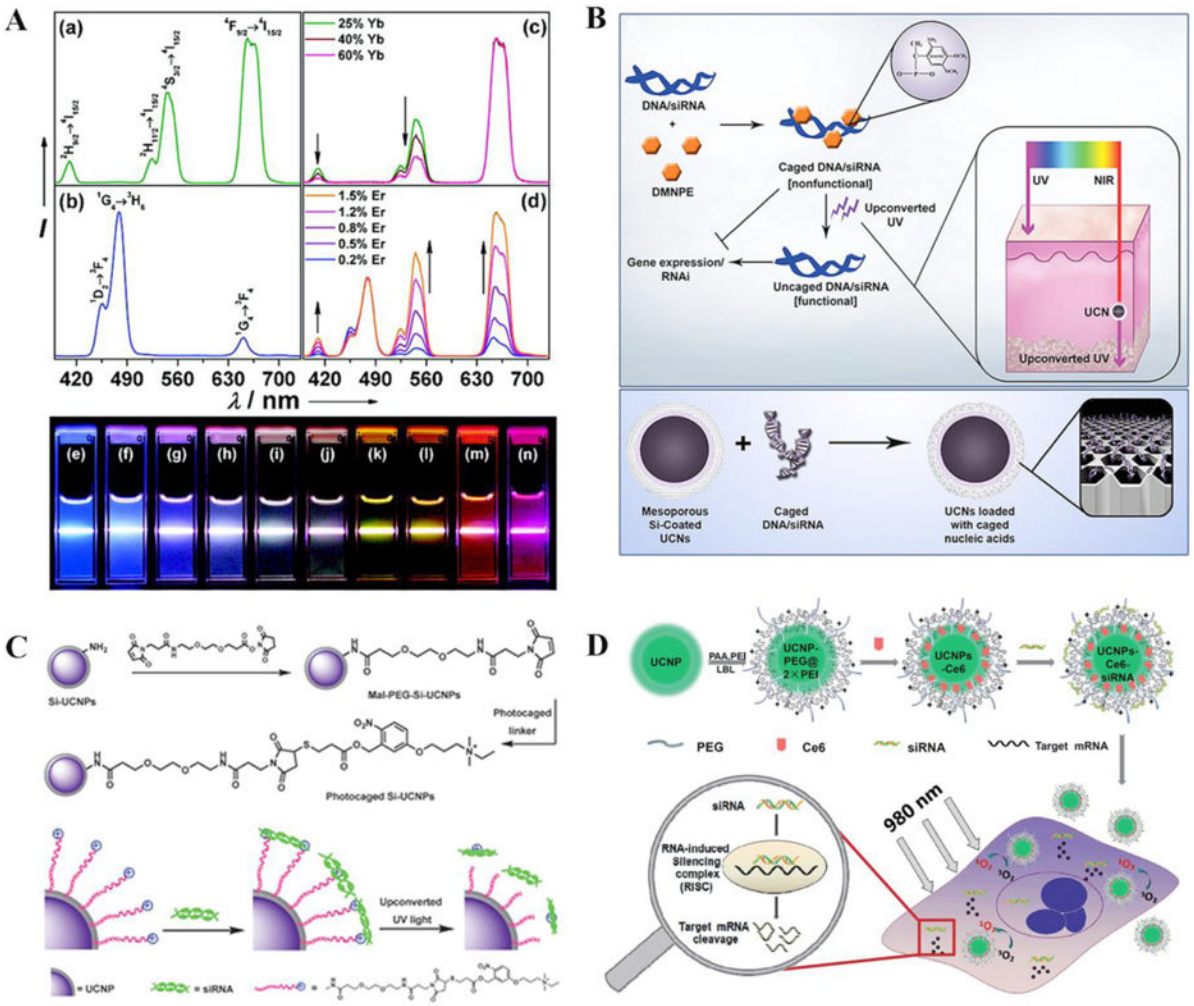

Fig. 9.

Hybrid UCNPs nanoformulated siRNA therapeutics. (A) The unique optical properties of UCNPs. (B) UCNPs for siRNA delivery and NIR light triggered siRNA activation. (C) UCNPs for siRNA delivery and NIR light activated siRNA release. (D) UCNPs for siRNA and photosensitizer Ce6 co-delivery for NIR-triggered photodynamic therapy combined with gene therapy for synergistically enhanced cancer cell killing effect. Figures reproduced with permission from (A) Ref. [162], (B) Ref. [175], (C) Ref. [176], and (D) Ref. [182]. Copyright (A) 2008 American Chemical Society, (B) 2012 National Academy of Sciences, (C) 2013 and (D) 2014 the Royal Society of Chemistry.

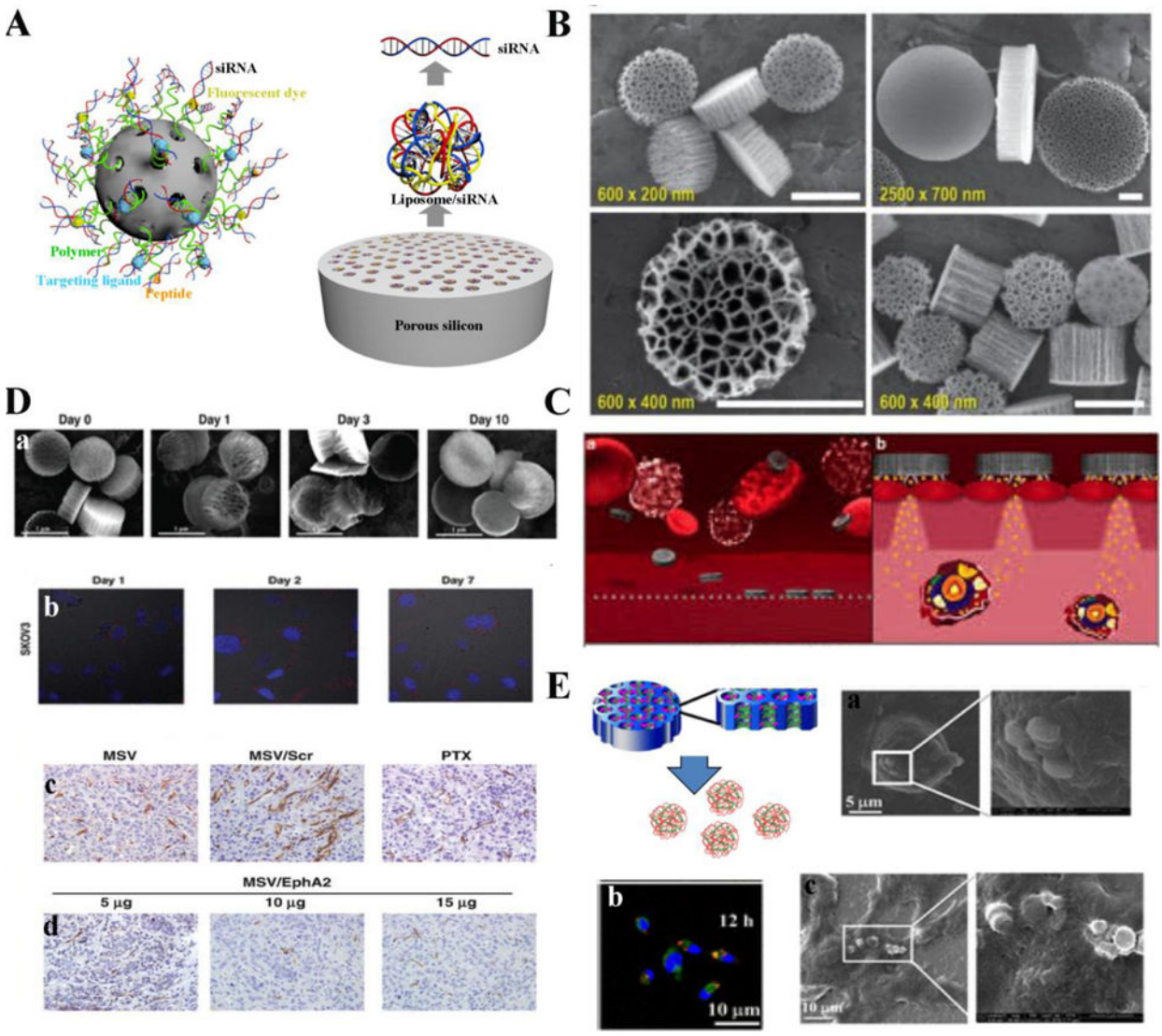

Fig. 10.

Hybrid silicon formulated siRNA therapeutics. (A) Schematic of silicon based nano/microparticles with different size and modification. (B) Scanning electron microscopy (SEM) images of unloaded MSVs with various dimensions and pore sizes. (C) The circulation routine and mechanism of MSVs. (D) Combination of MSV/EphA2 siRNA and paclitaxel for treatment of metastatic ovarian cancer. (E) Polycation nanoporous silicon (PCPS) releases self-assembled second nanoparticle for siRNA delivery. Figures reproduced with permission from (B) Ref. [197], (C) Ref. [195], (D) Ref. [198], and (E) Ref. [182]. Copyright (B) 2015 Elsevier B.V. (C) 2012 Springer Nature, (D) 2013 the American Association for Cancer Research, and (E) 2013 American Chemical Society.

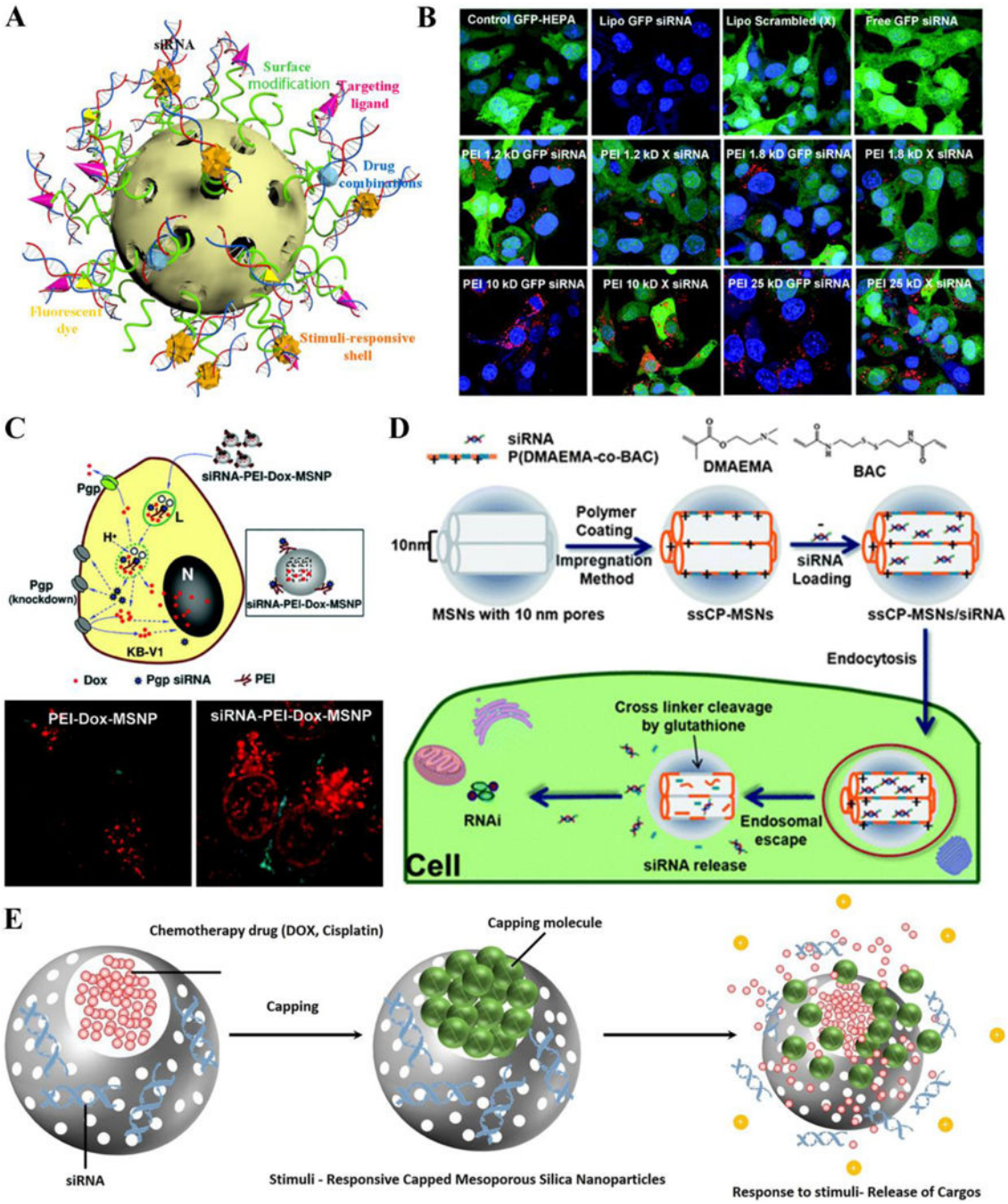

Fig. 11.

Hybrid silica nanoformulated siRNA therapeutics. (A) Schematic of multifunctional mesoporous silica nanoparticles (MSNs) for siRNA delivery. (B) MSN-PEI for siRNA delivery. (C) MSNs-PEI for co-deliver siRNA and doxorubicin (DOX) to treat the multiple drug resistance model (MDR). (D) Tumor microenvironment responsive MSNs system for siRNA delivery. (E) Tumor microenvironment responsive MSNs system for siRNA and DOX co-delivery. Figures reproduced with permission from (B) Ref. [216], (C) Ref. [220], (D)

Ref. [222], and (E) Ref. [225]. Copyright (B) 2009 and (C) 2010 American Chemical Society, (D) 2013 the Royal Society of Chemistry, and (E) 2017 Elsevier B.V.

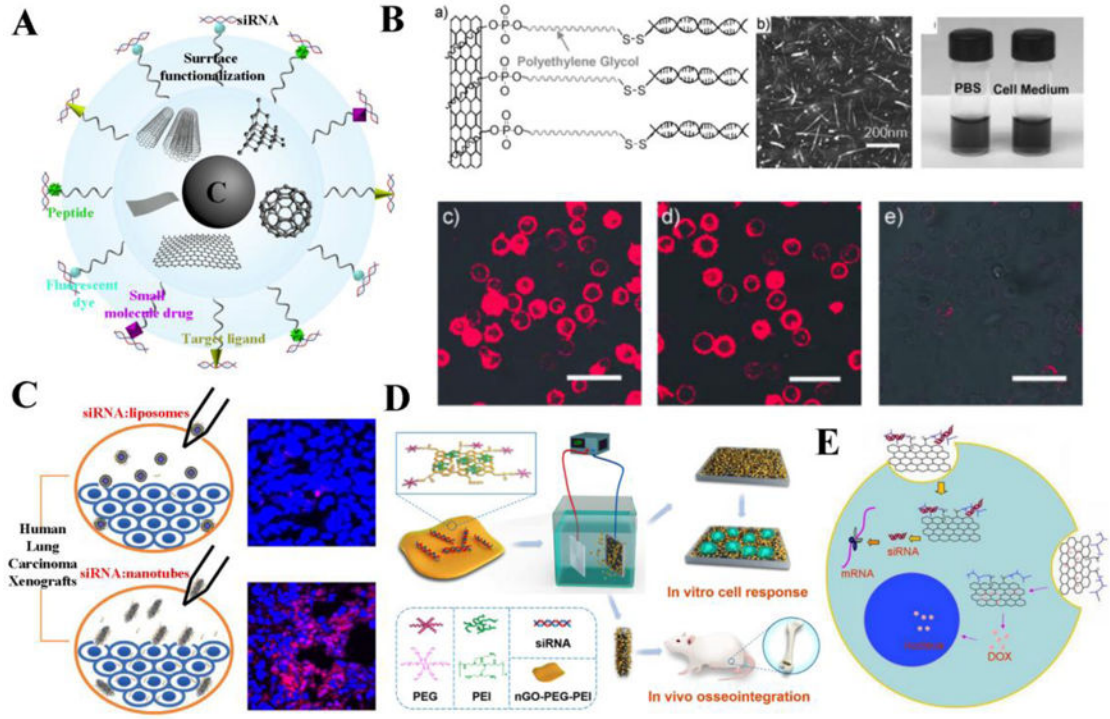

Fig. 12.

Hybrid carbon nanoformulated siRNA therapeutics. (A) Single-walled carbon nanotubes (SWNTs) for siRNA delivery. (C) Multi-walled carbon nanotubes (MWNTs) for siRNA delivery. (D) Graphene oxide system for siRNA delivery. (E) Graphene oxide (GO) system for co-delivery of siRNA and doxorubicin. Figures reproduced with permission from (B) Ref. [221], (C) Ref. [241], (D) Ref. [249], and (E) Ref. [251]. Copyright (B) 2007 and (E) 2011 John Wiley & Sons, (C) 2015 and (D) 2017 American Chemical Society.

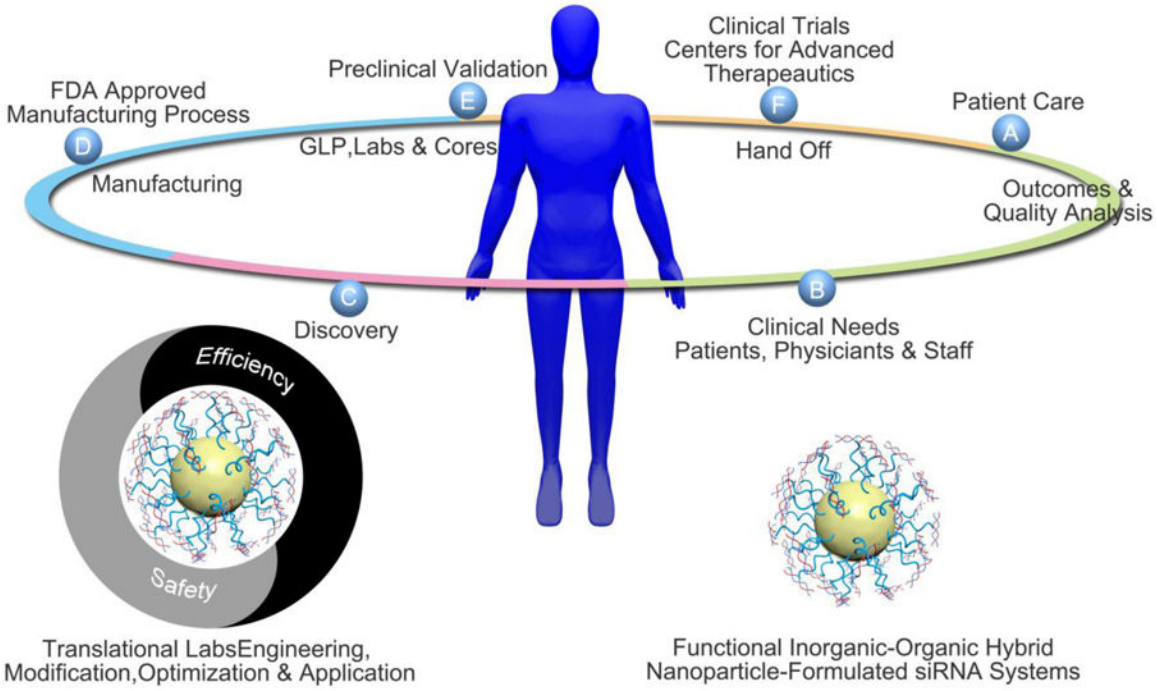

Fig. 13.

Inorganic-organic Hybrid systems formulated siRNA therapeutics for clinical needs.

Table 1

Examples of RNAi-based therapeutics in clinical trials for treatment of various human diseases.

Clinical subjects	Disease	Name	Gene	Delivery system	ClinicalTrials.gov Identifier	Phase	Ref.
Cancer	Advanced, recurrent cancer	siRNA-EphA2-DOPC	EphA2	LNP	NCT01591356	Phase 1	16
	Primary or Secondary Liver Cancer	TKM-080301	PLK1	LNP	NCT01437007	Phase 1	17
	Hepatocellular Carcinoma	DCR-MYC	MYC	LNP	NCT02314052	Phase 1/2	18
	Solid Tumors, Multiple Myeloma, or Lymphoma	DCR-MYC	MYC	LNP	NCT02110563	Phase 1	19
	Solid Tumor Cancers	CALAA-01	M2 subunit of ribonucleotide reductase (RRM2)	LNP	NCT00689065	Phase 1	20
	Pancreatic Ductal Adenocarcinoma; Pancreatic Cancer	siG12D LODER	KRAS	Polymer	NCT01188785	Phase 1	21
	Pancreatic Ductal Adenocarcinoma; Pancreatic Cancer	siG12D-LODER	KRAS	Polymer	NCT01676259	Phase 2	22
	Advanced Solid Cancer	Atu027	PKN3	LNP	NCT00938574	Phase 1	23
	Carcinoma, Pancreatic Ductal	Atu027	Protein kinase N3 (PKN3)	LNP	NCT01808638	Phase 1/2	24
Ocular and retinal disorders	Subfoveal Choroidal Neovascularization (CNV) Secondary to Age-Related Macular Degeneration (AMD)	AGN-745 (siRNA-027)	VEGFR-1	Naked siRNA	NCT00363714	Phase 1/2	24
	Choroid Neovascularization; Age-Related Macular Degeneration	AGN211745 (siRNA-027)	VEGFR-1	Naked siRNA	NCT00395057	Phase 2	25
	Optic Nerve Atrophy (Stratum I) and Acute Non-Arteritic Anterior Ischemic Optic Neuropathy (NAION) (Stratum II)	QPI-1007	Caspase 2	Naked siRNA	NCT01064505	Phase 1	22
	Moderate to Severe Dry Eye Disease	SYL1001	Transient receptor potential cation channel subfamily V member 1 (TRPV1)	Naked siRNA	NCT03108664	Phase 3	26
	Ocular Pain Dry Eye	SYL1001	Transient Receptor Potential Vanilloid-1 (TRPV1)	Naked siRNA	NCT01438281	Phase 1	27
	Ocular Pain; Dry Eye Syndrome	SYL1001	Transient Receptor Potential Vanilloid-1 (TRPV1)	Naked siRNA	NCT01776658	Phase 1/2	22
	Diabetic Macular Edema	Bevasiranib (Cand5)	VEGF	Naked siRNA	NCT00306904	Phase 2	28
	Macular Degeneration	Bevasiranib (Cand5)	VEGF	Naked siRNA	NCT00259753	Phase 2	22
	Age Related Macular Degeneration	Bevasiranib	VEGF	Naked siRNA	NCT00557791	Phase 3	29
	Choroidal Neovascularization; Diabetic Retinopathy; Diabetic Macular Edema	PF-04523655	RTP801	Chemically modified	NCT01445899	Phase 2	30
	Glaucoma; Ocular Hypertension	SYL040012	β-adrenergic receptor 2	Naked siRNA	NCT01227291	Phase 1/2	31
	Open Angle Glaucoma; Ocular Hypertension	SYL040012	β-adrenergic receptor 2	Naked siRNA	NCT02250612	Phase 2	32
	Ocular Hypertension; Open Angle Glaucoma	SYL040012	β-adrenergic receptor 2	Naked siRNA	NCT01739244	Phase 2	33
	Homozygous Familial Hypercholesterolemia	ALN-PCSSC	PCSK9	LNP	NCT02963311	Phase 2	34

Clinical subjects	Disease	Name	Gene	Delivery system	ClinicalTrials.gov Identifier	Phase	Ref.
Urinary system Disease	Atherosclerotic Cardiovascular Disease; Familial Hypercholesterolemia; Diabetes	ALN-PCSSC	PCSK9	LNP	NCT02597127	Phase 2	[34]
	Hypercholesterolemia	PRO-040201	apolipoprotein B	LNP	NCT00927459	Phase 1	[22]
	Delayed Graft Function in Kidney Transplantation	QPI-1002	pro-apoptotic gene p53	Naked siRNA	NCT00802347	Phase 1/2	[22]
	Primary Hyperoxaluria Type 1 (PH1)	ALN-GO1	glycolate oxidase (GO)	Naked siRNA	NCT02706886	Phase 1/2	[32]
	Primary Hyperoxaluria Type 1	DCR-PH1	HAO1	LNP	NCT02795325	Phase 1	NCT02795325
Others	Hypertrophic Scar	STP705	TGF-β1 and Cox-2	Polypeptide nanoparticle (PNP)	NCT02956317	Phase 1/2	NCT02956317
	Pachyonychia Congenita	TD101	K6a	Naked siRNA	NCT00716014	Phase 1	[22]
	Moderate to Extensive Fibrosis (METAVIR F3-4)	ND-L02-s0201	HSP47	LNP	NCT02227459	Phase 1	[18]
	Normal Healthy Subjects	ND-L02-s0201	HSP47	LNP	NCT01858935	Phase 1	[18]

Table 2

Surface engineering of representative inorganic nanoparticles through covalent ligand conjugation strategy

Nanoparticle	Anchoring groups	Ligands	Ref.
Au nanoparticle (AuNPs)	Thiol or disulfide	α-Acetal-ω-mercapto-poly(ethylene glycol), cysteine-terminated nuclear targeting peptides, thiol-containing dendron ligands, poly(N-isopropylacrylamide), thioctic acid-modified DNA oligonucleotide	42, 44–47
Quantum dots (QDs)	Thiol or disulfide	Thioctic acid, dithiocarbamate ligands, dihydrolipoic acid-zwitterionic ligands	48–50
	Carboxyl	Carboxymethyl chitosan, bicarboxyl dendron ligand, l-arginine-modified β-cyclodextrin	51–53
	Imidazole	Multidentate-imidazole polymer ligands	54
	Phosphine	Oligomeric phosphine ligand	55
Iron Oxide nanoparticle	Carboxyl	2,3-dimercaptosuccinic acid, carboxymethylated polyvinyl alcohol	56, 57
(IONPs)	Dopamine	Dopamine-nitrilotriacetic acid ligand, dopamine-poly(ethylene glycol), dopamine-biotin	43, 58, 59
	Phosphonate	Poly(ethylene glycol)-phosphonate	60
Upconversion nanoparticle	Carboxyl	Polyacrylic acid, poly(ethylene glycol)-diacid,	61, 62
(UCNPs)	Phosphonate	Poly(ethylene glycol)-phosphonate	63
	Amino	Poly(amidoamine), polyethylenimine	64, 65
Silicon/Silica nanoparticle	Various functional groups derived from silane ligands (carboxyl, amino, aldehyde, thiol, isocyanato, et al)	Poly(ethylene glycol), polyethylenimine, cyclodextrin, nuclear targeting peptide, et al	66–70
Carbon nanostructure	Surface carboxyl and hydroxyl groups	Poly(ethylene glycol), polyethylenimine, chloroacetic acid, hyaluronic acid, chitosan, et al	71–73

Table 3

Representative amphiphilic polymers used for hydrophobic inorganic nanoparticle surface engineering by non-covalent assembly method

Hydrophilic backbone	Hydrophobic chain	Nanoparticles	Ref.
Poly(acrylic acid) or PEGylated poly(acrylic acid)	Polybutylacrylate, polyethylacrylate, alkylamine (octyl, dodecyl, hexadecyl, octadecyl)	QDs, UCNPs	74, 76, 77
PEGylated poly(maleic anhydride)	1-octadecene, 1-decene, styrene	QDs, IONPs, Carbon nanotube, reduced graphene oxide,	75, 78–80
PEGylated poly(γ-glutamic acid)	Pyrene, phospholipid 1,2-distearoyl-sn-glycero-3-phosphoethanolamine	Carbon nanotube	75