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Review Article

Nanocarriers for siRNA Therapy

Fahima Dilnawaz*

Laboratory of Nanomedicine, Institute of Life Sciences, India

Abstract

Small interfering RNAs (siRNAs) therapy has opened exceptional advantageous opportunity for the treatment of diseases. Usually naked siRNA are subjected to internal harsh environment and rapidly degraded by RNases after administration. To overcome this issue various nanodelivery platforms have been developed and utilized in drug delivery due to narrowed size distribution, improved bioavailability and site specific target, impacting lowering of doses. To amenable the delivery of siRNA, various nanovehicles are designed and complexed with specific siRNA for protecting the siRNA payloads and avoiding nonspecific delivery. This review describes the recent developments of siRNA-complexed nanovehicles, which can probably carry forward to the next generation for their successful clinical translation.

***Corresponding author**

Fahima Dilnawaz, Laboratory of Nanomedicine, Institute of Life Sciences, Nalco Square, Chandrasekharpur, Bhubaneswar, Odisha, India, Tel: 674-2302-094; Fax: 674-2300-728; Email: fahimadilnawaz@gmail.com

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INTRODUCTION

RNA interference (RNAi) is the inherent machinery of cells for the regulation of specific gene expression which is precisely performed by double-stranded ribonucleic acid (dsRNA) that down regulates the expression with matching nucleotide sequence by obstructing or decaying mRNA translation [1]. As the commencement of dsRNA molecules blocked the function of the targeted gene hence the process was termed 'RNA interference'. Classes of small RNA such as endogenous microRNA (miRNA), exogenous small interfering RNA (siRNA), short hairpin RNA (shRNA) wield their silencing power differently [2]. The pathway of RNAi inside the cytoplasm is of two parts. The first part is the "initiation phase" where two effector molecules such as siRNA and miRNA are being formed. The duplex of siRNA is of 21-23 nucleotides created in cytoplasm starts through the splitting of long double-stranded RNA by the enzyme Dicer [3,4]. However the formation of miRNA is from nucleus, where the endogenous encoded primary miRNA transcripts (pri-miRNA) are processed into precursor miRNA (Pre-miRNA), thereafter in cytoplasm it is cleaved by Dicer. The second phase of it is the "effector phase" where both siRNA and miRNA are unwound and then assembled into RNA-induced silencing complex (RISC) [5]. Generally siRNA possess perfect complementary sequence, and miRNA typically have imperfect complementary that leads to translation repression without degrading mRNA. But both of them lead to inhibition of protein synthesis [6,7]. The heart of RISC is the Argonaute (AGO) proteins. Out of 8 AGO proteins, only the AGO2 is responsible for unwinding the siRNA [8]. Inside the RISC complex with the action of AGO2 protein the sense strand (passenger strand) is cleaved, and now the activated RISC contains one antisense strand (or guide strand) of siRNA or miRNA that guides RISC to the complementary or near-complementary region of target mRNA Figure (1). The cleavage of mRNA occurs between the nucleotide position of 10 and 11 of the complementary strand relative to 5′end, this process continues with activated RISC complexes and destroy additional mRNA targets further propagating the gene silencing process [5,9,10] this additional potency gives enormous scope for therapeutic effect for 3-7 days in rapidly dividing cells and several weeks in non-diving cell [11]. To achieve a persistent effect for longer period repeated administration of siRNA is necessary as the therapeutic threshold within the cells is diluted. This process can be repeated for several rounds of gene silencing process making it extremely useful strategy for therapeutic intervention [12,13]. siRNA being potent sequence-selective inhibitor it is being thought for powerful tool for modulating gene expression in any disease-causing gene as well as any cell type or tissue in various field. The silencing phenomenon by RNAi was first discovered with the injection of dsRNA into *Caenorhabditis elegans* that resulted in the silencing of complementary mRNA sequence [14]. Soon after, it was discovered in plants [15] and drosophila [16,17]. In mammalian cell line Elbashir et al., demonstrated the first proof-of principle experiment that exogenous siRNA could achieve gene knockdown [18]. Thereafter, gene silencing in mice was observed for hepatitis C [19]. Since that time siRNA has made considerable progress in the treatment of various diseases [20-22].

Although the therapeutic application of siRNA is extremely powerful as demonstrated with various *in vitro* and *in vivo* studies, but still it has many limitations of intracellular and extracellular barriers that needs to overcome to harness the full potential of this technology. Extracellularly the siRNA are highly unstable and susceptible to degradation of enzymes present in serum and tissues, so achieving an appropriate therapeutic level is a major challenge [23] in serum as well as in tissues [24]. In the cytoplasm they are also vulnerable to intracellular RNAses for degradation. Further the size, negative charge and hydrophilicity of naked siRNA prevents their diffusion across the plasma membrane and limits the intracellular accumulation. The translation of RNAi technology from an experimental approach to a clinical-mode of viable therapy for the benefits of patients,

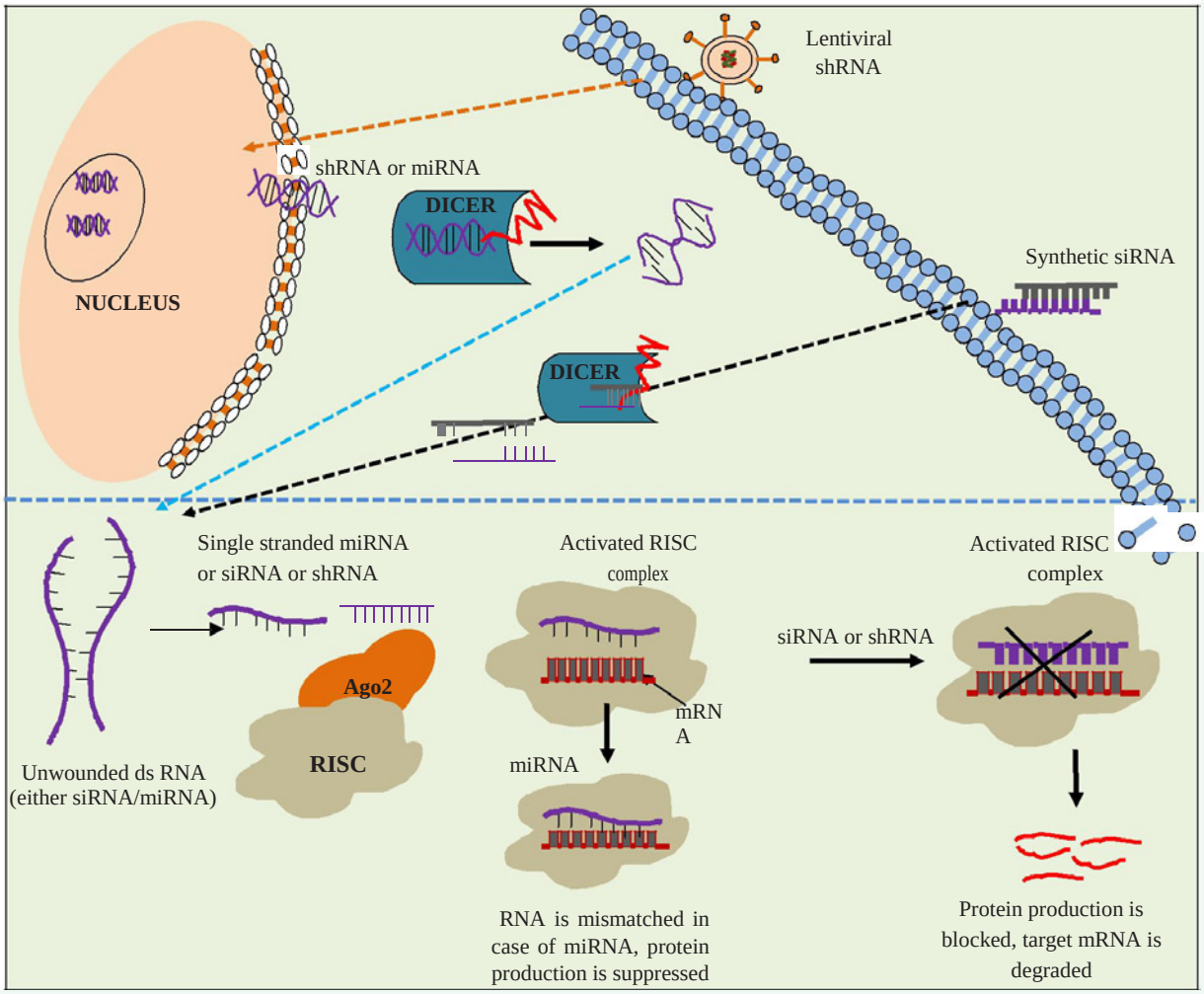

Figure 1 Schematic illustration of mechanism of RNA interference in gene silencing.

specific and efficient delivery systems are required. In order to achieve high transfection efficiency viral based vectors are used for siRNA delivery. In practice several gene therapy trials produced adverse effect and encountered various safety issues [25]. Apart from that, off target silencing effects by siRNAs also can lead to mutation of gene expression and unanticipated cell transformation, studies have shown that most off-target silencing usually occurs due to the homology of six to seven nucleotides in the "seed region" of siRNA sequence [26]. In some cases the intravenous injection of naked siRNA experiences fast renal clearance and potentiates immune response recognition through Toll-like receptors (TLRs) and trigger certain immune response of interferon [27,28]. For the therapeutic application through systemic delivery, the siRNA delivery system should confer prolonged circulation time, pinpointed reach to the targeted tissues and effectual cytoplasmic delivery to RISC etc. Therefore, it has become an immensely important task to develop a safe and effective nonviral siRNA delivery to overcome the challenges. In recent years nanotechnology driven nanomaterials have become the emerging platform for the delivery of effective RNAi therapeutics by giving adequate characteristics such as protecting the payload, precision intracellular delivery, enhancing the residence time along with guarding the siRNA delivery as well as overcoming the drug resistance [29].

Required properties of nanoparticles for siRNA delivery

The surface property of the nanoparticles significantly influences its way of interactions with the physiological molecules. In an *in vitro* setting uptake is facilitated by the positively charged delivery vehicle with negatively charged cellular membrane and the positive charge helps in complex formation and compression with the siRNA [30]. But the scenario is more complicated *in vivo* because of the presence of negatively charged serum proteins in blood streams which often binds to the positively charged nanoparticle, thus making it ineffective. To surmount this problem the coating with polyethyelene imine (PEI) or polyethylene glycol (PEG) assists is usually preferred. Addition of PEG preferably reduces the particle size and particle aggregation in the serum, evading the immune system and phagocytosis [31]. Furthermore by forming barrier around the nanoparticles PEG provides the steric stabilization and protection from physiological condition [32]. This review focuses on various types of synthetic materials that have been studied for the delivery of siRNA both *in vitro* and *in vivo*.

Biomaterials for siRNA delivery

Synthetic biomaterials have potentially demonstrated its usefulness as effective non-viral siRNA carriers. Various commonly used nanodelivery vehicles that are used for siRNA delivery are polymeric nanoparticles, polymeric micelles, liposomes, dendrimers etc; based gene delivery systems (Figure 2).

Polymeric nanoparticles

Linear and branched chain cationic polymers PEI act as effective transfection agents due to their ability to condense and stabilize nucleic acids [33-35]. The complex formed between the cationic polymer and nucleic acid is known as polyplexes. Better transfection efficiency of the PEI vector is its ability to avoid the trafficking to lysosome, principal barrier of gene transfer. Mostly popular hypothesis of high transfer is due to the "proton sponge effect". The PEI results additional pumping of protons inside the endosome that leads to the accumulation of chloride ions for maintaining the charge neutrality and increase of ionic strength. This ground for the osmotic swelling and physical rupture of endosome, thus facilitating the vector's escape from

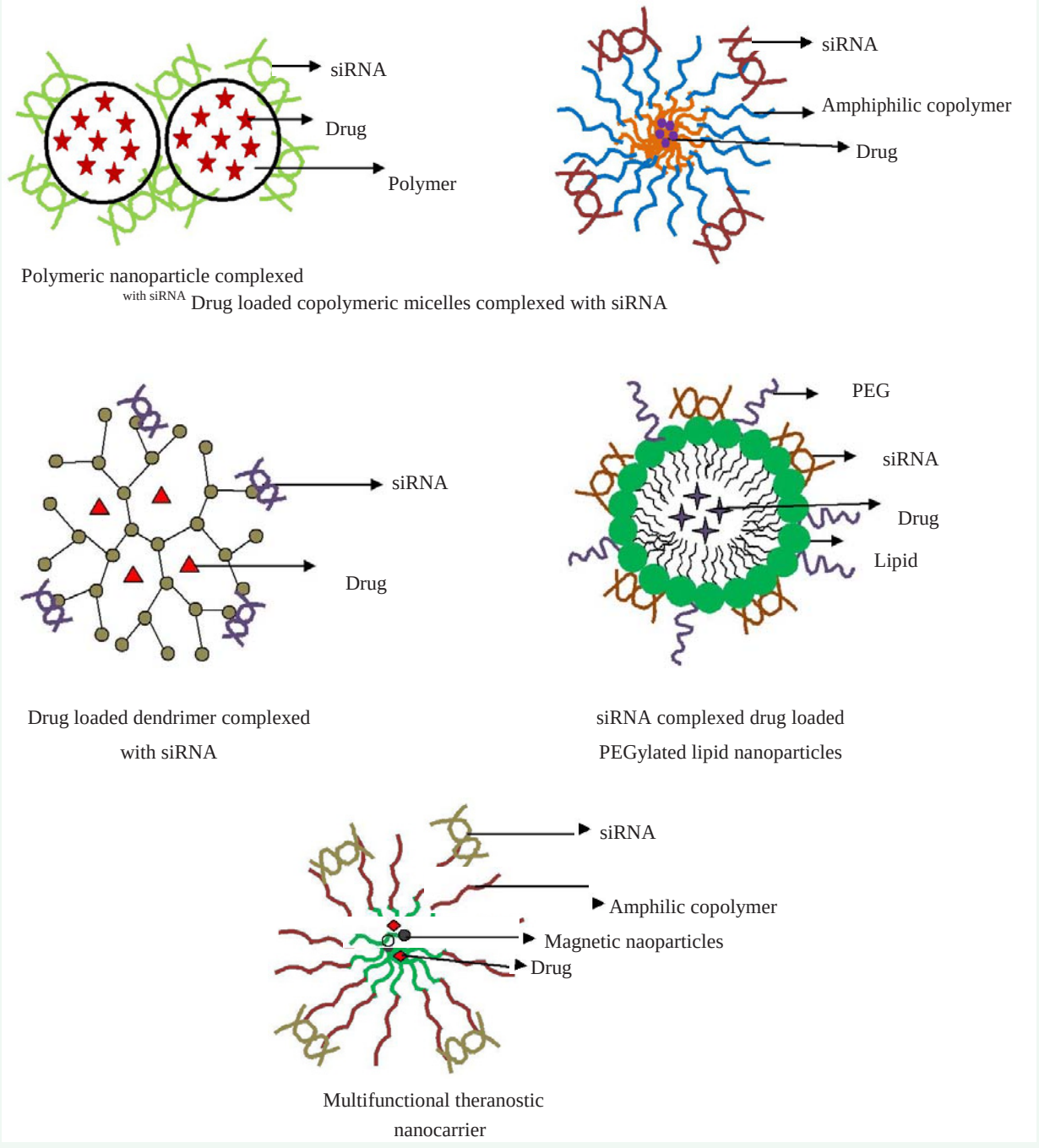

Figure 2 Various nanocarriers used for delivery of siRNA.

the degradative lysosomal trafficking pathway [36,37]. Urban-Klein et al., reported efficient delivery of siRNA-PEI complexation into cells displaying full bioactivity in mouse tumor model. The intraperitoneal (i.p.) administration of PEI-complexed HER-2-specific siRNAs resulted reduction of tumor growth by down regulating HER-2 in SKOV-3 xenografts, however injection of naked HER-2 siRNA could not exert inhibitory effect on growth [38]. Cyclodextrin polymers are also developed for the siRNA delivery, because their terminal imidazole group assists in intracellular trafficking and release of nucleic acid [39]. The cyclodextrin-containing polycations (CDP) was mixed with adamantane (AD)-PEG, and the polyplexes was targeted with transferrin-modified AD-PEG and siRNA for *EWS-FLI1* was added for the generation of stable targeted particles. The nontargeted particles CDP and AD-PEG are combined and added to siRNA to generate stable but nontargeted polyplexes. The murine model of metastatic Ewing's sarcoma received formulation of cyclodextrin bound siRNA against the *EWS-FLI1* gene product through tail vain. There was a noticeably inhibition of growth in the murine model of metastatic Ewing's sarcoma [40]. Knockdown was not observed with control siRNA and nonconjugated formulation which significantly eliminates the antitumor effect and no evidence of immune stimulation nor abnormality or toxicity was observed, providing evidence of safety and efficacy of the nonviral siRNA delivery system [40].

Davis reported regarding the efficacy of cyclodextrin-based nanoparticles containing PEG (CALAA01 siRNA) formulation for targeted delivery. The formulation was designed to knockdown RRM2, and a transferring receptor targeting protein [41]. Using the formulation by systemic administration phase-1 clinical trial was conducted to the patients of solid tumours. The melanoma patient were administered the formulation and the post treatment tumor biopsies showed presence of intracellularly localized nanoparticles with reduction in both the specific messenger RNA (M2 subunit of ribonucleotide reductase (RRM2) and the protein (RRM2) levels compared to pre-dosing tissue. Fragments of RRM2 mRNA from patients sample indicated induction of desired mRNA cleavage. This study corroborated specific gene inhibition through siRNA in human patients by an RNAi mechanism [42].

Polymer–siRNA conjugates have also shown potential for applications in systemic siRNA delivery, in this connection Rozema et al., have developed dynamic polyconjugates for delivery of siRNA to hepatocytes to treat metabolic disorders, *in vivo* after low-pressure i.v. injection. These are membrane active cationic polymer that has the ability to reversibly mask the activity of this polymer until it reaches the acidic endosomal environment. The siRNA is targeted for two endogeneous genes of liver apolipoprotein B (apoB) and peroxisome proliferator-activated receptor alpha (ppara). There was significant decrease of serum cholesterol and increased fat accumulation in liver. Knockdown of ppara also resulted in significant reduction of *ppara* mRNA levels in liver. The synthetic polymer is well tolerated by mice as analysed with serum liver enzyme and cytokine levels [43]. Huh et al., designed polymeric nanovehicle containing glycol chitosan (GC) and PEI, and were conjugated with 5β-cholanic acid for stabilization and -homing ability. These cationic charged nanovehicles were complexed with negatively charged red fluorescence protein (RFP) gene silencing siRNA aimed to inhibit RFP expression. In *in vitro* study using RFP expressed B16F10 tumor cells revealed enhanced time-dependent cellular uptake with siRNA–GC–PEI nanoparticles. There was significantly inhibition due to higher -targeting ability of RFP gene expression in RFP/B16F10-bearing mouse models demonstrating the vectors ability for siRNA delivery [44]. siRNA vascular endothelial growth factor (VEGF) was used to study the tumor suppressive growth and metastasis. Chitosan-siRNA-VEGF nanoplexes along with Neuropilin-1(co-receptor of VEGF) was injected intraally into the breast-bearing Sprague-Dawley rats. The tumor volume was measured after 21 days, where marked reduction in tumor volume was observed illustrating the suppressive activity of VEGF expression and volume in breast cancer model of rats [45].

Recently, Wang et al., developed galactosylated chitosan-graft-poly(ethylene glycol) (GCP) nanoparticle for delivery of Polo-like kinase 1(PLK1) siRNA nucleotides into hepatocellular carcinoma cells. The GCP-siRNA complex inhibited the cell proliferation due to depletion of PLK1 and induced apoptosis along with impaired tumorigenicity *in vivo* [46]. Arami et al., developed polyacrylate-based cationic nanoparticles and complexed with survivin siRNA for knowing down the anti-apoptotic genes. The nanoparticle complex consisted of Fe_3O_4 and layered with polyacrylate (PA) and polyethyleneimine (PEI). The siRNA-Fe_3O_4-PA-PEI nanoparticles demonstrated the effective delivery of siRNA into the cytoplasm of MCF-7 cells with induction of apoptosis [47].

Xu et al., designed pH-sensitive co-delivery system for delivering doxorubicin and survivin siRNA by pulmonary administration. Conjugates of polyethylenimine (PEI) with doxorubicin (DOX) were developed via a pH-sensitive hydrazine bond (3-maleimidopropionic acid hydrazide, BMPH) (PEI-BMPH-DOX (PMD). The drug release was increased from PMD conjugates with decreasing of pH values. In the B16F10 tumor-bearing mice models, the pulmonary delivery of PMD-siRNA complex preferentially accumulated drugs and siRNA in the tissues of lung, but very less amount of drug was observed in normal lung tissues. With this there was enhanced antitumour activity with nanoparticulate formulations than that of mono-delivery of drug or siRNA. The pulmonary delivery approach could be an effective treatment approach for metastatic lung cancer [48].

Hypoxia sensitive polymeric carriers are designed to exploit to respond to the gradient oxygen tension inside the tumor. Perche et al, reported first of its kind for the down regulation of GFP gene through hypoxia activation. A hypoxia-responsive azobenzene was used bio-reductive linker. The nanocarrier was formed using polyethylene glycol 2000-azobenzene-polyethyleneimine 1.8 kDa-1,2-dioleyl-sn-glycero-3-phosphoethanolamine (PAPD). The PAPD carrier demonstrated higher hypoxia- dependent cellular uptake that resulted in elevated down regulation of GFP in a number of GFP expressing cells under hypoxic conditions. To know the gene silencing effect the bearing mice of A2780/GFP was administered PAPD/siRNA i.v and down regulation of GFP was observed through *ex vivo* imaging as well as by flowcytometry [49].

Polymeric Micelles

Over the past few years micelle-from amphiphilic block

copolymers have been used for siRNA delivery, its efficacy are enhanced with attaching the targeting ligand and incorporating sensitive blocks into them. Christie et al developed multifunctional micelles from block copolymer PEG-b-poly (l-lysine) (PEG-b-PLL), where a thiol group is introduced for attaching the siRNA, the lysine is modified with 2-iminothiolane (2IT) for increased stability, along conjugation of cRGD peptide on PEG terminus for improved delivery both *in vitro* and *in vivo*. To reduce the blood supply to the siRNAs for vascular endothelial growth factor (VEGF) receptors were used. Christie et al, developed multifunctional polymeric micelle for siRNA against VEGF (to target tumor mass) and siRNA VEGFR2 (targeting blood vessel endothelial cells), another targeting ligand cyclo-Arg-Gly-Asp (cRGD) peptide, was incorporated for the enhanced tumor accumulation, cellular uptake distribution. Following i.v injection to the mice, enhanced gene silencing ability and improved accumulation in the tumor mass and tumor-associated blood vessels exhibited effective inhibited growth of subcutaneous HeLa-Luc tumors and silenced genes in the tumor mass. With naked siRNA and micelle lacking cRGD peptide no tumor growth reduction was observed [50]. Combinational approach for cancer has been proven effective; in this regard micelles are also developed to address the therapy. Sun et al, developed innovative "two-in-one micelleplex from biodegradable triblock copolymer poly (ethylene glycol)-b-poly (ε-caprolactone)-b-poly (2-aminoethyl ethylene phosphate) for systematic delivery of siRNA and drug. Using triblock copolymer paclitaxel drug was encapsulated and conjugated with siRNA specific to polo-like kinase 1 (Plk1) to study the inhibitition of cancer. The micelleplex could efficiently knockdown the expression of the therapeutic target gene Plk1 (key regulator of mitotic progression in mammalia) whose activity is elevated in cancer. The simultaneous delivery of paclitaxel and knockdown of gene induce synergistic suppression in the MDA-MB-435s xenograft murine model, with minimal requirement of drug compared to mono therapy of paclitaxel without triggering the innate immune response.

For treatment of malignant glioma modified poly (ethyleneglycol) and poly (caprolactone) and a cell penetrating peptide (TAT) [M-PEG-PCL-TAT] micelle was used for co-delivery of camptothecin drug and siRNA against Raf-1 (which plays a role in cell proliferation and apoptosis) and tested against a rat model. The cell death was promoted in glioma cells due to higher cellular uptake of siRaf-1 by the micelleplex carrier MPEG–PCL–TAT and the mean survival period was increased compared to untreated rats. The co-delivery of drug and siRNA in micellar complex led to increased therapeutic efficacy in CNS diseases [51]. Micelles constructed by matrixmetalloproteinase2 (MMP2)-sensitive copolymer polyethylene glycol - peptide -polyethylenimine-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine and was loaded with paclitaxel drug and conjugated with siRNA specific to survivin. Incubation of cells with micelle complex enhanced the cellular uptake *in vitro* leading to higher cytotoxic activity compared to native paclitaxel drug. Further simultaneous delivery of siRNA complexed micelles, the surviving protein was down regulated and was dose dependent and *in vivo* co-delivery studied on a Non-small-cell lung carcinoma xenograft mouse model also corroborated the efficacy of the targeted delivery of the siRNA micelle complex [52]. Xiong and Lavasanifar, reported the efficacy of multifunctional micellar system from poly (ethylene oxide)-block-poly (ε-caprolactone) (PEO-b-PCL) block copolymers with functional groups on both blocks. The micelles prepared from this block have the versatility that can stably complex siRNA conjugate doxorubicin, imaging probe and having the ability to have virus like shell for specific cell recognition and cellular uptake. These micelles can release the drug by pH triggered mechanism. The RGD/TAT micelles having siRNA and Doxorubicin (Dox) could efficiently demonstrate significant cellular uptake, better drug penetration and superior cytotoxicity in doxorubicin resistant cells and oncogene-silencing effects [53]. With the help of redox-sensitive micelles siRNA can be delivered effectively. Matsumoto et al., reported about the polyion complex (PIC) micelles that was made through the assembly of iminothiolane-modified poly (ethylene glycol)-*block*-poly (L-lysine) [PEG-*b*-(PLL-IM)] and later complex with siRNA (against Pp-Luc). This siRNA complexed micelle demonstrated higher transfection efficacy into the target cell and gene silencing effect compared to non-cross-linked PICs prepared from PEG-*b*-poly (L-lysine). The disulfide groups are cleaved in the cytoplasm and provide stability and prompt release of the cargo molecules inside the target cell [54]. Self-assembled cationic biodegradable polymeric micelles were prepared with diblock copolymers of linear PEI and PCL (PEI-PCL). For co-delivery of drug and siRNA these micelles were loaded with Dox and siRNA (antiaopototic Bcl-2) along with targeting ligand folic acid (FA). The FA was first conjugated to poly (ethyleneglycol)-block- poly (glutamic acid) (FA-PEG-PGA) and thereafter coated electrostatically to PEI-PCL micelle that was preloaded with siRNA and Dox. This micellar complex resulted high transfection efficiency as well as controlled release of Dox potentiated the cell death through synergistic effect *in vitro* [55]. The *in vivo* effect was studied in a rat model having *in situ* C6 glioma implant, administration of folate targeted drug siRNA micellar complex at the right striatum of tumor induced rat significantly down regulated the Bcl-2 gene and up regulated pro apoptotic gene Bax, further activating caspase-3 subjecting the apoptosis effect, further leading to prolong survival of the rat [56].

Polymeric Dendrimers

Dendrimers are branched macromolecules with designed architecture to bear functionalized groups. Kala et al, developed triethanolamine (TEA)-core poly (amidoamine) (PAMAM) dendrimers having primary amines dendrimer surface and the branching units have tertiary amines. Advantage of this dendrimer is that they self-assemble with siRNA into compact, nanoparticulate form by electrostatic interactions and in turn siRNA is protected. The siRNA dendrimer complex was evaluated in SKOV-3 drug resistant ovarian cell line, where Akt play a major role in cell proliferation and survival. After the introduction of G6-mediated delivery of Akt siRNA there was reduction at the protein expression level depicting the gene silencing effect when compared to G6 alone and siRNA alone. Combination of paclitaxel drug and AktsiRNA-dendriplex, there was significant growth inhibition of SKOV-3 cells, compared to only drug and only siRNA dendriplex alone. For tumorigenicity study Akt siRNA dendriplexes was administered intraperitoneally to SKOV-3 subcutaneous of mice. There was reduction of outgrowth significantly in Akt siRNA/G6-treated mice compared to the

naked siRNAor G6 dendrimer treated mice after one week. Dendrimer-mediated Akt siRNA delivery, along with drug constitutes an effective approach for cancer therapy [57]. Serramia et al utilized cationic carbosilane dendrimer of second generation and tested its therapeutic efficacy for targeting the Nef expression for the reduction of HIV-1 infectivity. The cellular uptake and distribution of dendrimers and dendriplexes into BALB/c mice by injecting them to the retro-orbital venous plexus. The 2G-$(SNMe_3I)_{11}$-FITC dendrimer was transported efficiently siRNA into the brain crossing the *in vivo* BBB model validating the potentiality of carboxysilane dendrimer for gene therapy [58].

Lipid nanocarriers

Lipid nanocarriers are advantageous in nature because of the natural tendency of cell interaction with the cell membrane facilitating the cellular uptake of RNA. Furthermore, there is relatively lower risk of undesirable immunogenic reactions to lipids. Several lipid based nanocarriers such as liposomes, lipid-based nanoparticles and lipid nanoemulsions are used for the siRNA delivery. Chen et al., developed both cationic liposome-polycation-DNA (LPD) and anionic liposome-polycation-DNA (LPD-II), for systemic co-delivery of drug and siRNA. The developed formulation demonstrated high loading efficiency of drug and siRNA. Conjugation of siRNA c-Myc and siRNA-VEGF along with doxorubicin significantly enhanced the growth inhibition of ovarian tumor cells with respective gene silencing effect [59]. Lipid nanoemulsions are developed for anticancer theranostic (therapy+ diagnostics). For which hydrophobic paclitaxel is dissolved in lipiodol (iodinated poppy seed oil) and emulsified with a mixture of PEGylated phospholipids, cholesterols, and linear PEI attached with cholesterols and electrostatically complexed with Bcl-2 siRNA. The cationic nanoemulsion demonstrated elevated level of cytotoxicity and apoptosis due to synergistic activity in breast adenocarcinoma compared to individual treatments. Preliminary investigation of lipidol nanoemulsion in mouse model illustrated the feasibility of bio-imaging with micro-computed tomography, suggesting it for a multifunctional lipid nanocarrier for theranostics [60]. Leukocytes, the body gate-keepers and cellular constituent of immune system are hard to transfect. Efforts have been taken to silence the genes in lymphocytes using siRNA for various disease treatments. Modulation of T cell function by RNA interference (RNAi) holds tremendous potential in down regulating specific genes. Recently, Ramishetti et al developed lipid nanoparticles comprised of the fusogenic ionizable lipid Dlin-MC3-DMA to transfect natıve $CD4^+$T lymphocytes using CD45 siRNA. To study the *in vivo* silencing 8 week C57BL6/J mice were injected with siRNA (CD45)-lipid nanoparticles and after 5 days the mice were sacrificed and collected for analysis and it was found that the mice treated with siRNA has the CD45 silencing effect in leukocytes over the control groups validating the high specificity [61]. Wei et al. synthesized lipid nanoparticles with Dlin-KC2-DMA and its analogs to transfect in adherent cells. Incorporation of siRNA-kinesin family member-11 (KIF11) to lipid nanoparticles could efficiently deliver it to mouse hematopoietic tissues of spleen and bone marrow in nude mice. The group has also demonstrated that the transfection efficiency is mostly dependent over the expression of Caveolin 1 and 2 backed by microarray analysis [62]. The treatment of blood cancer has been explored by siRNA mediated lipid nanoparticles. Mantle cell lymphoma (MCL), aggressive B cell lymphoma which over expresses cyclin D1 and has poor prognosis, down regulation of cyclin-D1 is a potential therapeutic target. Weinstein et al has formulated lipid nanoparticles composed Dlin-MC3-DMA and functionalized and entrapped antibody (CD-38) and siRNA (cyclin D1) respectively. The above functionalized lipid nanoparticles induced effective gene silencing in mantle cell lymphoma (MCL) cells *in vitro*. MCL lymphoma bearing xenografted mice when treated with CD38-targeted lipid nanoparticles that contain cyclin D1 siRNAs, after i.v induced cell specific gene silencing that prolonged the survival of the mice, demonstrating a new therapeutic opportunity for the B-cell malignancies [63].

Cancer treatment, takes the advantage of enhanced permeability and retention (EPR) effect, which is attributed to immature vasculature. Sakurai et al., developed lipid nanoparticles that are complexed with endothelial cell-targeting siRNA, evaluated its intratumoral distribution and therapy. After administration the VEGFR2 was inhibited by liposomal siRNA, which results improved intratumoral distribution and effective therapeutic efficiency regardless of maturity of tumour vasculature. When intervened with small inhibitor molecule of matrix metalloproteinase and macrophage, the distribution mode of lipid nanoparticles gets affected demonstrating the EPR effect is dependent on structure of tumour vasculature as well as dynamics of tumor microenvironment and extracellular matrix, thus by regulating these factors the potentiality of the EPR based approach could be further enhanced [64].

Gujrati et al have designed dual pH-sensitive lipid-siRNA self-assembly nanoparticles for encountering the oncogene associated drug resistance and potentiating the efficacy of the chemotherapy. siRNA of eukaryotic translation initiation factor 4E (eIF4E), is complexed to the RGD-PEG (HZ)-ECO. siRNA was delivered effectively both *in vitro* and *in vivo* because of dual pH sensitivity. i.v injection of RGD-PEG(HZ)-ECO/siRNA nanoparticles (1.0 mg-siRNA/kg) to the MDA-MB-231 tumor bearing mice demonstrated gene silencing for one week. However in athymic nude mice administration of RGD-PEG (HZ)-ECO/sieIF4E every 6 days for 6 weeks down regulated the over expression of eIF4E and resensitizes the paclitaxel drug to the resistant MDA-MB-231 tumors with minimum immunogenicity [65].

Recently, effectiveness and advantages of nanoparticle based drug delivery systems in the clinic has been reported by Anselmo and Mitragotri [66]. Most of the clinical trials of gene therapy are liposome based. Especially few clinical based studies on liposome gene therapy for cancer are completed and some have entered the clinical trial [67,68]. There are few examples of current clinical trials such as SGT-53. The SGT-53 is a cationic liposomal therapeutic complex comprising of plamid encoding normal human wild-type (wt) p53 DNA. The liposomal complex is functionalized with single-chain antibody fragment (scFv), the anti-transferrin receptor (TfR) to target cancer. As the transferrin glycoprotein receptor are ubiquitous and overexpressed in tumor cells. Aiming to target the p53 dysfunction and restoring its proper function is one of the ways to treat cancer. After administration the liposomal complex

specifically delivers the p53 cDNA to tumor cells both in primary and metastatic via receptor mediated endocytosis [69,70]. Moreover, in preclinical studies the intravenous administration of SGT-53 liposomal complex demonstrated significant tumor growth inhibition and regression in solid tumors when used in combination with chemotherapy and radiation [71]. After the success this technology was advanced for human clinical trials. In another lipid nanoparticle based therapeutic approach was used for knocking down the oncoprotein (MYC) that was untreatable with standard therapies [72]. Liposomal siRNA formulation (Atu027) was developed which targets protein kinase N3 (against the oncogene c-MYC), causal gene for malignant cell growth. Early clinical study on patients with advanced solid tumours showed limited cytokine activation. Atu067 treatment was well tolerated and the disease was stabilized in 41 % patients, the plasma samples of the patients showed increase in siRNA anti-strand concentration [73,74]. Other than cancer clinical trials are also focused for iron replacement or imaging etc. Hepatitis B is treated by knocking down three genes in the hepatitis B genome by liposomal siRNA (ARB-001467) effectively facilitating the knockdown of the viral mRNA transcripts and antigens [75]. In another study, gene therapy was used for the treatment of hepatic fibrosis [76]. Sato et al developed vitamin-A coupled liposomes for delivering siRNA against gp46, responsible for fibrogenesis as well as uptake and storage of vitamin A. Administrating siRNA-bearing vitamin A-coupled liposomes to rats completely resolved the liver fibrosis and prolonged the survival of rats [76]. For the treatment of familial amyloid polyneuropathy (FAP) progressive, fatal disease caused by deposition of transthyretin (TTR) liposomal siRNA therapeutic was used for knockdown of the TTR protein. Early clinical trials showed 94 % after the treatment and 77 % after 28 days [77,78]. These types of treatments are impactful and provide a cure and relief to patients. Various nanoparticulate based drug delivery vehicles which are currently undergoing clinical trials are listed (Table 1).

Theranostic nanocarriers

Magnetic nanoparticles (MNPs) that are from iron oxide particles have shown tremendous ability for clinical translation in drug delivery and imaging as well as exclusive MRI study. In the amphiphilic PDMA-b-PCL diblock copolymer, SN-38 drug and MNPs were encapsulated. This drug and MNPs loaded micelles were then complexed with VEGF siRNA-PEG via electrostatic attraction between the anionic moiety of siRNA and cationic moiety of PDMA-b-PCL micelles. The cytotoxicity activity was evaluated in colon cancer cell line LS174T (having high VEGF expression). The VEGF siRNA micelleplex exhibited good silencing effect compared to free VEGF siRNA as it showed poor suppression because of degradation. The *in vivo* anticancer activity of drug loaded MNPs micelleplex was studied in nude mice that with subcutaneously induced LS174T xenografts. Administration of free VEGF siRNA exhibited suppressive effect on growth because of instability and enzymatic degradation. The significantly inhibited growth was observed in multidose of VEGF siRNA drug loaded micelleplex displayed synergistic therapeutic effect of gene silencing as well as anticancer activity. Further the MRI image (T2-weighted) of the micelleplex mice on day 7 illustrated significant inhibitions by the designed multidose treatment compared to control mice. The developed theranostic micellar system has the capability of gene silencing effect as well as contrast agent for monitoring of the tumor [79]. In another study Lee et al developed multimodal carrier which can simultaneously deliver and imaging using MNPs. The developed multifunctional carrier can accurate image by delivering the therapeutics which inturn can minimize the invasiveness and deleterious side effects. The core is of manganese-doped magnetism-engineered iron

Table 1: Nanoparticle based siRNA therapies which are currently undergoing various clinical.

Company	Nanocarrier	siRNA targets	Disease types	Clinical trials.gov identifier (phase)
Arbutus Biopharma	Lipid	Polo-like kinase 1 (PLK1)	Hepatocellular Carcinoma	NCT02191878 (Ph I/II)
Arbutus Biopharma	Lipid	Target three sites on HIV genome	Hepatitis B	
M.D. Anderson Cancer Center	Lipid	ephrin type-A receptor 2 (EphA2)	Solid tumors	NCT01591356 (Ph I)
Dicerna Pharmaceuticals	Lipid	Oncogene (Myc)	Solid tumors, multiple myeloma, lymphoma, or hepatocellular carcinoma	NCT02110563 (Ph I) NCT02314052 (Ph I/II)
Nitto Denko	Lipid	Heat shock protein 47 (HSP47)	Hepatic fibrosis	NCT02227459 (Ph I)
Alnylam Pharmaceuticals	Lipid	Transthyretin (TTR)	Amyloidosis	NCT02510261 (Ph III) NCT01961921 (Ph II) NCT01960348 (Ph III)
Alnylam Pharmaceuticals	Lipid	Kinesin spindle protein (KSP) and vascular endothelial growth factor (VEGF)	Solid tumors	NCT01158079
Senesco Technologies	Polymer	eIF5A^{K50R}plasmid eIF5A siRNA	Myeloma, Lymphoma, Leukemia	NCT01435720 (PhII)
Silence Therapeutics GmbH Silenseed	Lipid Polymer	Atu027 PKN3 knockdown in vascular Kirsten Rat Sarcoma (KRAS)	Pancreatic cancer Pancreatic cancer	NCT01808638 (Ph I/II) NCT01188785 (Ph I) NCT01676259 (PhII)

oxide coated with bovine serum albumin (BSA). The amine group of BAS was converted to pyridyl disulfide groups by treating with Nsuccinimidyl- 3-(2-pyridyldithio) propionate (SPDP), which is further treated with thiolated PEG functionalized with cyclic Arg-Gly-Asp (RGD) peptide, known to bind specifically to $\alpha_v\beta_3$ integrin. The obtained nanoparticle was treated with Cy5-dyelabeled thiolated siRNA (Specific to GFP) (HS–siGFP–Cy5). The advantage of linkage of siRNA through disulfide bonds is that it could cleave readily in intracellular environment. *In vitro* study was carried out in endogenous GFP cells MDA-MB-435-GFP and A549- GFP. The feature of the multimodal nanoparticles, where the PEGylated RGD provides stabilization and targeting, and the siGFP-Cy5 is responsible for gene silencing and fluorescence imaging. MRI image guides siRNA delivery *in vitro* and *in vivo* gives potential qualitative information about the diseased tissues [80]. Kumar et al, reported about the ability of the synthesized magnetic nanoparticles for dual targeting purpose by conjugating Peptide (EPPT, targets uMUC-1) and complexing siRNA antiapoptotic gene BIRC5 to target human breast tumors. This designed magnetic nanodrug complex uptake is enhanced that significantly downregulated the BIRC5. In subcutaneous mouse model of breast cancer after i.v administration the nanodrug complex were preferentially uptaken by the tumor tissues, visualized by MRI and near-IR optical imaging. The i.v injection once in a week over two weeks induced drastic reduction of tumor growth, depicting active targeting ability as well as the gene silencing effect. With the help of MRI the bioavailability of the drug is monitored throughout the course of treatment, illustrating the ability for theranostic application [81]. Li et al developed CdSe/ZnSe quantum dot (QD) based nanocarriers for the lung cancer therapy. The QD-nanocarriers were surface conjugated with l-arginine (l-Arg) and different kinds of hydroxypropyl-cyclodextrins (HP-α-CDs, HP-β-CDs, and HP-γ-CDs). The QD nanocarriers were further loaded with different anticancer drug and siRNA (target Bcl-2). The utilization of QD nanocarriers along with different anticancer drugs there was 3 to 4 fold increase of cytotoxicity in A549 cells due to combined action of drug and gene silencing. Because of the fluorescence property these QD nanocarriers could be used as theranostic purpose providing real time imaging [82].

CONCLUSION AND FUTURE PERSPECTIVES

The ability of siRNA to silence the particular gene of interest has open up new avenues for the therapy of many diseases. Nanoparticulate delivery vehicles for siRNA received maximum gene silencing effect and have emerged as one of the successful approach by lessening the adverse effect. In future further new polymeric delivery vehicles would be developed for specific targeted manner to carry siRNA leading to precision medicine.

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