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Applications of Lipidic and Polymeric Nanoparticles for siRNA Delivery

Behiye Şenel and Gülay Büyükköroğlu

Abstract

The antisense technology that emerged with the discovery of RNA interference nearly 20 years ago has gained a significant place in gene therapy. siRNA, one of two important components of RNA interference, efficiently downregulates gene expression in human cells, so it has the potential to eradicate disease. siRNA delivery systems, which can be applied both systemically and locally in different diseases, have gained significant importance. Naked small RNAs can be delivered directly to cells, but because of their instability, exposure to enzyme degradation, and difficulties in reaching/entering the target cell or tissue in blood stream, these initiatives are failing. For this reason, the method of delivery or encapsulation of siRNA is usually required. Various nanoparticles, nanocapsules, emulsions, micelle systems, metal ion nanoparticles, and nanoconjugates have been used for siRNA delivery. In these transport systems, lipidic and polymeric systems are very attractive due to their advantages such as being biodegradable and biocompatible, safety, being able to electrostatically bind to RNA, long-term stability, well-illuminated structure and features, simple and easy production, etc. Issues such as particle size, zeta potential, and stability of siRNA-loaded system should be taken into consideration in the development of siRNA delivery systems.

Keywords: lipidic nanoparticles, polymeric nanoparticles, nanocarrier, transfection, barriers, siRNA delivery

1. Introduction

Noncoding RNAs have many functions such as gene silencing, DNA imprinting, and demethylation. An increased number of noncoding RNAs have been discovered in gene regulation and RNA processing. Of these, small interfering ribonucleic acid (siRNA) and microRNAs (miRNAs) that can interfere with the translation of the target mRNA transcript are small ncRNAs that are the cleavage products of the dsRNA. MicroRNAs are a class of endogenous RNA that results in mRNA translation inhibition or degradation, which regulates cell differentiation, proliferation, and survival, while synthetic siRNA can initiate the silencing of target genes without interrupting natural mRNA pathways.

siRNA is a promising therapeutic solution preventing gene overexpression in various pathological conditions such as infectious or ocular disease, cancer, and genetic or metabolic disorders. These therapeutic methods are currently being a

phenomenon in cancer therapy because siRNA is used to suppress oncogenes and to remove mutations and to illuminate key molecules in the cellular pathways of cancer [1, 2].

It is also effective in personalized gene therapy for several diseases, due to its specificity, compatibility, and targeting ability. Following this strategic discovery, many synthetic siRNAs are designed with the desired sequences to explicitly inhibit any target gene expression [3, 4]. However, naked siRNA is unstable in the bloodstream and cannot effectively pass the cell membranes as well as being immunogenic [5].

There is a need for delivery systems in order to overcome the obstacles and to increase their potentials in the process of transporting siRNAs to the desired destination with minimal adverse effects and safe transportation to target areas. These carriers are usually provided with viral (retrovirus, lentivirus, adenovirus, adeno-associated virus (AAV), cough viruses, human foamy viruses, and baculovirus) and non-viral vectors. Viral vectors are effective tools for transfection vectors which are efficient delivery systems that utilize genetically modified viruses, offering advantages such as continuous gene silencing and the ease of expression of a large number of RNA interference (RNAi) molecules from a transcript [6, 7].

However, safety concerns such as oncogenicity, immunogenicity, viral genome insertion into host chromosomes, and expensive production limit the widespread use of viral vectors [8].

Until now, only one virus-mediated transport system has been authorized for marketing. Glybera® (alipogene tiparvovec using an adeno-associated virus as vector) was the first drug approved by the European Commission in late 2012 to treat lipoprotein lipase deficiency (LPLD). However, in early 2018, the most expensive drug in the world has been withdrawn from the market for financial reasons [9]. Therefore, it is important to design non-viral transport vectors for the in vivo delivery of siRNAs.

These delivery systems are mostly intended to make the siRNA more efficient for interaction with angiogenesis, metastasis, chemoresistance of tumors, and proliferation of cancer cells. Although these systems show encouraging results for possible therapeutic success, many obstacles still exist to implement them in humans, practically.

Delivery systems should not be immunogenic and should not cause undesirable side effects. In normal cells, siRNAs should avoid off-target silencing of genes [10].

The carrier systems should not be defined as foreign particles by the immune system elements, i.e., interferons and cytokines, and should not be eliminated before they reach the target. These difficulties have been overcome by the precise design of the siRNA to the target or by chemical modification of the relevant siRNA molecule.

For this reason, the use of lipidic/polymeric nanoparticles is the most common choice to overcome the above difficulties. These developed nanoparticles can protect the siRNA from plasmatic nucleases and unwanted immune responses, thereby facilitating endocytosis, designed to resist renal clearance; providing low cytotoxicity, stable serum stability, and high structural and functional reliability; delivering the unstable naked siRNA to targeted tumor sites; and reducing interactions with nontarget cells [7].

They can also be used for on-target delivery by adding target-specific ligands to their surface. The most preferred among these transport systems are lipid-based and polymer-based systems. Several technologies and nanoparticle modifications developed in recent years and new nanoparticle raw materials have accelerated the development of siRNA-loaded nanoparticles with the desired structural and functional properties.

DRUG name	Disease or condition	Target	Delivery vector	Phase	Trial number	Company	Recruitment status
CALAA-01	Solid tumors	RRM2	CD/polymer	1	NCT00689065	Calando Pharmaceuticals	Terminated 2008–2013
ALN-VSP02	Solid tumors	KSP, VEGF	LNP	1	NCT00882180	Alnylam Pharmaceuticals	Completed 2009–2011
Atu027	Advanced solid tumors	PKN3	LNP	1	NCT00938574	Silence Therapeutics GmbH	Completed 2009–2013
PRO-040201	Hypercholesterolemia	ApoB	SNALP	1	NCT00927459	Arbutus Biopharma Corporation	Terminated 2009–2010
TKM-080301	Primary or secondary liver cancer	PLK1	LNP	1	NCT01437007	National Cancer Institute (NCI)	Completed 2011–2018
DCR-MYC	Hepatocellular carcinoma	MYC	LNP	1b/2	NCT02314052	Dicerna Pharmaceuticals, Inc.	Terminated 2014–2018
	Solid tumors, multiple myeloma, lymphoma	MYC	LNP	1	NCT02110563	Dicerna Pharmaceuticals, Inc.	Terminated 2014–2017
siRNA-EphA2-DOPC	Advanced solid tumors	EphA2	NL	1	NCT01591356	M.D. Anderson Cancer Center	Recruiting 2012–2018
ND-L02-s0201	Moderate to extensive hepatic fibrosis	HSP47	LNP	1	NCT01858935	Bristol-Myers Squibb	Completed 2014–2017

KSP, kinesin spindle protein; VEGF, vascular endothelial growth factor; PEI, polyethylenimine; NP, neutral liposome; CD, cyclodextrin; PKN3, protein kinase N3; PLK1, polo-like kinase-1; ApoB, MYC oncogene, apolipoprotein B; SNALP, stable nucleic acid lipid particle.

Table 1.
siRNA-based clinical trials using lipidic and polymeric vectors for cancer therapy [12, 13].

Taking advantage of these excellent properties of nanoparticles, various delivery systems for siRNA have recently used the clinical trial phase, and these methods are followed as a very effective and promising treatment for various disease.

As the latest development in this issues, the US Food and Drug Administration approved the Onpattro (patisiran) infusion on 10 August 2018 for the treatment of peripheral nerve disease (polyneuropathy) caused by hereditary transthyretin-mediated amyloidosis (hATTR) in adult patients. It is also the first FDA approval of a new class of drugs called small interfering ribonucleic acid (siRNA) treatment. FDA anticipates that this is the beginning of a new and exciting generation of therapeutics [11].

Patisiran is covered in a lipid nanoparticle (LNP) that carries the drug to the liver, but this carrier molecule can trigger its own immune response. Therefore, steroid, acetaminophen, and antihistamines should be used to reduce the likelihood of immune reaction in patients before taking patisiran administered by intravenous (IV) infusion. Further research is underway to design a drug delivery system that can reach the target tissue without causing an immune response. **Table 1** presents examples of clinical trials and current status of siRNAs prepared with lipidic and polymeric nanoparticle.

2. Physiological barriers for siRNA

The site of action of the SiRNA therapeutics is cytosol. The obstacles to siRNA administration vary depending on the targeted organs, disease, and routes of administration. In general, siRNA therapeutics are preferred as local or systemic injection. The local transport of siRNA has less obstruction compared to systemic administration. The first of the unique features of the local administration is the need to an easy formulation that requires easier production and application and secondly provides application of lower doses that limit intracellular (time-concentration-dependent) immune responses [14].

Therefore, the application is limited to easily accessible tissues such as the skin, eye, or mucosa, but successful results have been achieved. However, for the local administration of siRNA, the nervous system, lung, digestive, vagina, and inner coronary walls were selected as target regions [15].

For example, intranasal inhalation of the siRNA against the respiratory syncytial virus was carried out with TransIT-TKO commercial transfection agent and was found significantly effective in reducing viral infection [16].

In a recent study by Wu and colleagues, they developed novel PEGylated lipoplex-entrapped alginate scaffold system for vaginal epithelium-targeted siRNAs for the treatment of HIV virus infections. They indicated in the study the potential of the biodegradable PLAS system for the sustained delivery of siRNA/oligonucleotides to vaginal epithelium [17].

The main advantage of the systemic method is rapid action and biodistribution but also a wide range of applications. In addition, intravenous or intraperitoneal (IP) injection therapy is successfully applied for many diseases in the clinic. However, it causes significant difficulties for siRNA in systemic transmission. The transition steps of the siRNA from the application site to the target action site are shown in **Figure 1**.

As described above, after intravenous injection (1), siRNA is distributed to organs through the blood stream (3) and also undergoes elimination [7].

The siRNA molecules have unwanted physicochemical properties due to their negative charge, large molecular weight, size, and instability. After systemic administration, the naked siRNA administered without vectors is rapidly degraded by serum endonucleases (4), excreted by the kidney (7), or taken up by the macrophages (APC cells) (5) of the mononuclear phagocyte system (MPS) [7, 18].

The result is a short plasma half-life below <10 minutes, so it cannot have enough time to reach the target and/or perform its functions, and therefore the gene silencing is ineffective. These problems are partially addressed by chemical modifications of the RNA backbone and by the use of nano-sized carriers (2) [7, 60].

Nanoparticle studies showed that the nanoparticles below 6 nm were rapidly excreted by renal excretion and nanoparticles in the range of 150–300 nm were taken by the cells of the MPS in the liver and spleen. It is stated that nanoparticles between 30 and 150 nm can hold in the bone marrow, kidney, etc. In addition, it has been reported that red blood cells (RBCs) affect the transport and distribution of microparticles in human microvasculature (diameter <100). In fact, due to collisions with RBCs, microparticles are pushed toward the artery wall where RBCs are virtually absent depending on the shape and size by a radial movement called the “waterfall effect.” This event is also called margination, and they have stated that the extravasation cannot occur unless vectors are exposed to margination [14].

In a study done by D’Apolito et al. [20], they found that 3- μm particles had better marginalization than 1 μm . For this reason, it is seen that the size of the prepared particles could be very effective especially in in vivo experiments.

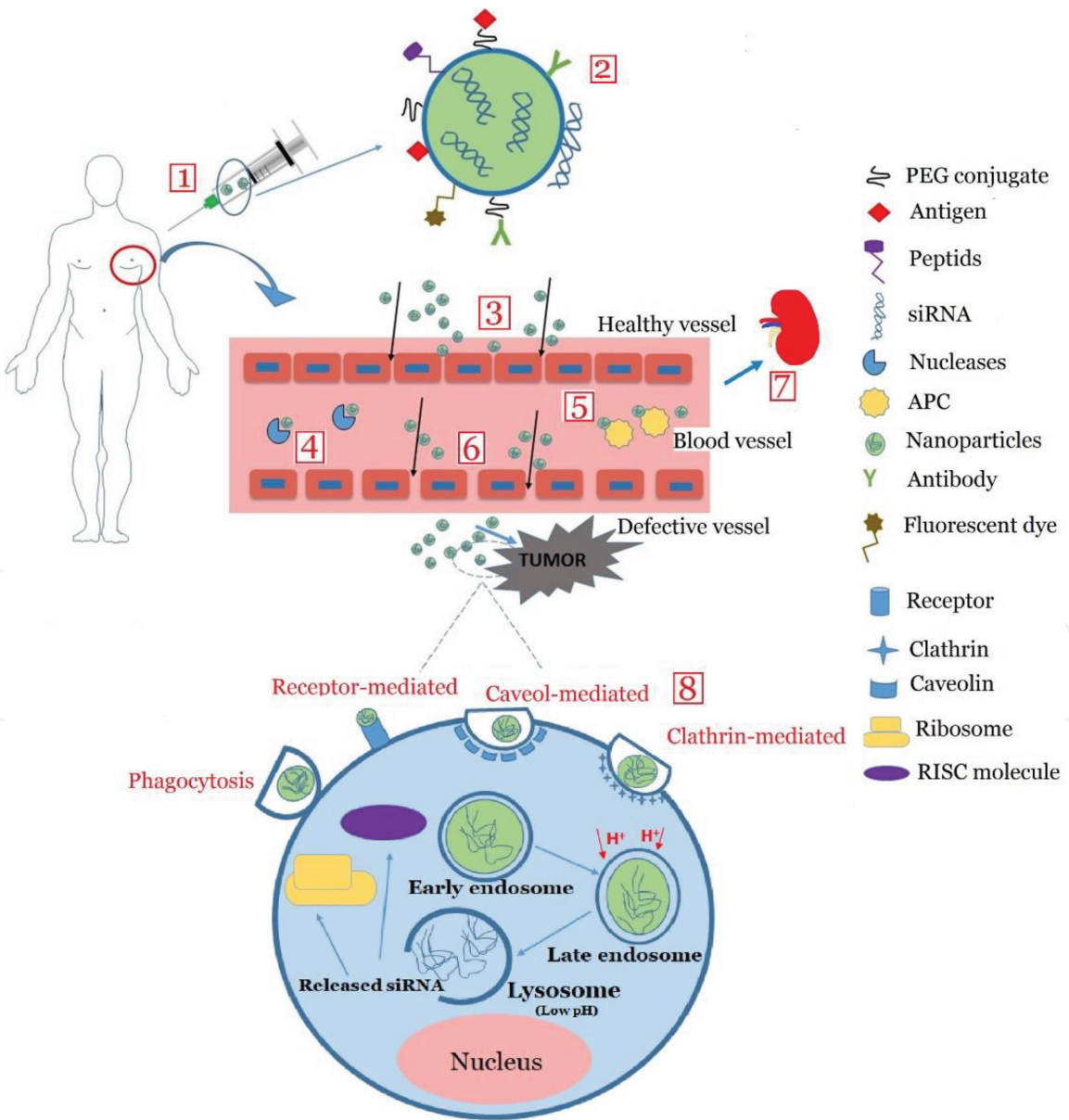


Figure 1.
An illustration of a nanocarrier, barriers at systemic circulatory, mechanisms of uptake of siRNA from cell (modified from the 26th literature). In the following text, the numbers in the figure are mentioned.

The vectors carrying siRNA enter the tissue interstitium following extravasation. After entering the tissue interstitium, siRNA is moved to the target cells across the interstitial space. Transport along the vessel walls can occur via diffusion, convection through the capillary pores, and transcytosis. The diffusion is directed by the concentration gradient.

One of the unique properties of tumor microvessels is leakage (6) from endothelial discontinuity. The pore size of tumor microvessels ranges from 100 to 780 nm in diameter. In contrast, microvessels in most normal tissues have less leakage. For example; the tight junctions between endothelial cells are generally <2 nm, and the pore size in the capillary venules is <6 nm, the endothelium of the renal glomeruli is 40–60 nm, and the sinusoidal endothelium in the liver and spleen has large pore sizes of about 150 nm [19].

Leakage in tumor vessels increases siRNA/carrier extravasation, since the naked siRNA cannot enter the direct cell membrane due to its high molecular weight, large size, and negatively charged phosphate skeleton from the anionic cell membrane; the carrier systems are gaining importance here, again [21, 22].

There is an evidence that endocytosis (8) plays an important role, although not all of the entry mechanisms are well understood. The most common endocytosis used by nanocarriers is clathrin-mediated endocytosis through endocytic route receptor [23].

Rejman et al. [24] showed that the size and nature of the carrier vector affect the internalization mechanism. Nanoparticles of about 1 μm size are taken up via macropinocytosis, and ~120 nm size are taken up via clathrin-mediated endocytosis, whereas nanoparticles ~90 nm are taken up via clathrin/caveolae-independent endocytosis and caveolin-mediated endocytosis [25, 26].

Besides surface properties and size of the nanoparticles, surface modifications of the nanoparticles are also important in siRNA transport. The most important of these surface modifications is the coating of nanoparticles with PEG. The PEGylation is performed to keep the particles in the systemic circulation longer, and the positive results were obtained from until today. However, in addition to this positive effect, it has been reported that the increased PEGylation from 1–2 to 5% mol reduced the transfection efficiency by neutralizing the positive surface load required for siRNA involvement instead of increasing the transfection [27].

After reaching the target cell, the siRNA is subjected to endocytosis internalization, a process involving encapsulated siRNA in endocytic vesicles fused with endosomes. Endocytotic vesicles are initially associated with early endosomes and then mature late endosomes before fusion with lysosomes in the cell. Lysosomes are then formed. After internalization into the cell, the siRNA should escape from the fragmentation in the endosomes and be released from the carrier to the cytosol in order to be loaded to RNA-induced silencing complex (RISC) [28].

Sardo et al. found that endosomal escape increased with another modification with pH-sensitive polymers. In this study, they prepared a siRNA delivery system based on inulin (Inu), a plenty and natural polysaccharide. Inu was functionalized via the conjugation with diethylenetriamine (DETA) residues to form the complex Inu-DETA. The results of the study showed that while homogenous diffusion of siRNA was performed in JHH6 cytoplasm via micropinocytosis and clathrin-mediated endocytosis, it was found that it did not allow caveola-mediated passage and no siRNA activity [29].

3. Types of nanoparticulate delivery systems for siRNA delivery

This section focuses on the biodegradable non-viral lipids and polymeric nanosystems used in the transport of synthetic siRNA. These nanoparticles used as

drug delivery vehicles are carriers having a particle size of <100 nm. They are composed of different biodegradable materials, such as natural or synthetic polymers, lipids, or metal.

The main advantages of nanoparticles used as drug carriers are achieving appropriate particle size, using of physiological lipids (e.g., triglycerides) and organic polymers (e.g., chitosan) to reduce immunogenicity, stimulating interferon- γ production and natural killer (NK) cells, activating antitumor immunity to increase the effectiveness of treatment, prolongation of blood circulation in blood, and feasibility of variable routes of administration; they can be viewed and monitored by marking.

However, they have some disadvantages; for example, the substances used during the formulation and the preparation methods may cause the increase of toxicity, sometimes failing to carry the siRNA into the cell or insufficient accumulation at the target site, and problems in the stability of lipidic structures like liposomes. These limitations, however, can be overcome by selecting the appropriate lipid or polymers. The advantages thus outweigh the disadvantages [30].

3.1 Lipid-based nanoparticle delivery systems

Lipid-based siRNA delivery systems include liposomes, micelles, microemulsions, ionizable lipids and lipid nanoparticles, and solid lipid nanoparticles.

3.1.1 Liposomes

Liposomes are spherical vesicles consisting of an aqueous core together with a bilayer phospholipid structure which contain natural body components (e.g., lipids, sterols) and are biologically compatible and biodegradable. Furthermore, liposomes are popular siRNA carriers due to their relative simplicity and well-known pharmaceutical properties. The amphipathic nature of liposomes allows the use of a wide range of hydrophilic and hydrophobic drugs. The hydrophilic molecules show a greater affinity between the hydrophilic head groups of the phospholipid bilayer and the aqueous core of the liposomes, while the hydrophobic molecules intercalate between the fatty acyl chains of the two lipid layers [31].

As analogues of biological membranes, liposomes are fused with the plasma membrane and are processed by endocytosis, and the genetic material is released into the cytoplasm. Cationic liposomes form complexes with negatively charged anionic siRNAs and polycations, and the complex is called as a “lipoplex.”

However, due to their positive charge, cationic liposomes can lead to dose-dependent cytotoxicity and inflammatory response, and the complexes can interact nonspecifically with negatively charged serum proteins. In order to solve these problems, successful tests were taken in preclinical studies of EphA2-targeted siRNA therapeutic using neutral lipids such as 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), which proved to effectively reduce cellular toxicity [31].

Several strategies have been implemented to overcome the disadvantages of existing lipid-based systems. Modification of the lipid structure or formulation methods can reduce toxicity and improve transfection. The inclusion of fusogenic lipids, such as dioleoylphosphatidylethanolamine (DOPE), or the use of a cationic lipid consisting of biodegradable ester bonds such as DOTAP may retain toxic effects at a relatively short or moderate level and also may increase the endosomal release of siRNA [32].

3.1.2 Solid lipid nanoparticles

SLNs having a size range of 50–1000 nm were composed from various lipids which are solid form at body or room temperature and can be stabilized with

surfactant or surfactant mixture. SLNs consist of a lipid core surrounded by a surfactant layer in an aqueous dispersion [33].

While being among the most effective carriers for both hydrophilic and hydrophobic drugs, such as liposomes, the solid, lipophilic nucleus of SLNs may have difficulty carrying RNA molecules that are hydrophilic and polyanionic. Therefore, it can be used successfully for gene delivery by addition of cationic lipids to SLNs which provide a positive surface potential [34].

SLNs can be easily prepared by various methods such as hot or cold homogenization, sonication, solvent evaporation, etc., and also they have high physical stability and low cytotoxicity [35].

Studies with siRNAs that have been adsorbed or encapsulated into SLNs have also shown positive results in the literature. For example, Şenel et al. [36] pointed out that Bcl-2-targeted siRNAs encapsulated into SLNs prepared by sonication method showed an activity to be able to compete with Lipofectamine in the liposomal form commercially used.

3.1.3 Nanostructured lipid carriers (NLCs)

Nanostructured lipid carriers (NLCs) are new second-generation lipid carriers that combine the advantages of different nanocarriers. These are solid lipid-core-modified SLNs in which the lipid phase may comprise solid or liquid forms at ambient temperature [37].

Compared to SLNs, NLCs have greater loading capacity and less water in the dispersion, making them more stable for storage. There was no difference in biotoxicity. Studies have shown that NLCs can be used as a new delivery tool for the genetic treatment of disease. Taratula et al. prepared a multifunctional NLC system containing two siRNAs against cellular resistance to doxorubicin or paclitaxel targeting lung cancer cells. It has been found that the system successfully increases the antitumor activity of the anticancer drug [38, 39].

NLCs can also be modified to achieve targeting and sustained release. For example, by manipulating the degradation times of NLCs, it has made possible to obtain long-acting siRNAs. This design raised the continuous release of siRNAs to 9 days (with a A-tailored nanostructure carrier design delivering survivin-siRNA); this also facilitates clinical practice of siRNA treatment [40].

3.1.4 Ionizable lipids and lipid nanoparticles (LNPs)

Recently, new lipid types have been proposed to the delivery of RNA interference molecules. Advanced LNP siRNA systems are lipid-based particles with diameters <100 nm. These may consist of a mixture of an ionizable amino lipid (e.g., heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate, DLin-MC3-DMA), a phosphatidylcholine, cholesterol, and a coat lipid (polyethylene glycol-dimiris glycerol) in a 50:10:38.5:1.5 molar ratio [41].

These lipids developed for the encapsulation of negatively charged genetic materials exhibit pH-dependent charge change. These lipids are positive at acidic pH and are neutral at physiological pH. Thus, under acidic conditions, nucleic acids are encapsulated in the nanoparticle and have a minimum positive charge density in the bloodstream. LNP siRNA systems are produced by rapidly mixing the lipids in ethanol with the siRNA in aqueous buffer (pH 4.0) followed by removal of ethanol via dialysis against PBS buffer after the pH is raised to 7.4 [42].

The genetic material carrier formulations generated by ionizable cationic lipids have been shown to be of significant success in in vivo activity after the initial administration in hepatocytes. Other tissues used for gene silencing include

macrophages, osteoclasts, and osteoblasts in the “hard” bone and distal tumor cells [43, 44].

Other siRNA administration methods have used lipid-like molecules called “lipidoids” for the delivery of siRNA. Lipidoid delivery systems are similar to ionizable cationic LNP systems due to the use of lipid-like molecules, cholesterol, and PEG-lipid. The most important difference of LNPs using lipidoid is that the lipidoid molecules have an extra positive charge because they have a large number of protonizable amine bound to various acyl chains. Similar to studies with cationic lipids, lipidoid systems developed by screening programs have been described in the literature [45].

Ball et al. developed a potent and nontoxic lipidoid nanoparticle (LNP) for intestinal epithelial cells. In the initial studies, it was reported that GAPDH siRNA-loaded LNPs for Caco-2 cells mediated strong, dose-dependent, and resistant gene silencing with a single 10-nM dose for 1 week [46].

3.2 Polymer-based delivery

Polymers of natural and synthetic origin have been used for various biomedical applications including drug targeting, imaging, gene therapy, prostheses, tissue engineering, etc. Because of their reproducible properties in terms of molecular weight, degradation, and mechanical properties, synthetic polymers are attractive for therapeutic applications. The most commonly used polymers include polyethyl- enimine (PEI), PLGA, PEG, PLL, PLA, etc. However, the synthetic polymers have the disadvantage biologically, such as they can turn into undesirable side effects or fail to achieve the desired bioactivity and biocompatibility. On the other hand, natural polymers are abundant and are similar to components of those found in biological extracellular matrices. Thus, the natural polymers have high bioactivity and biocompatibility. Natural polymers include polysaccharides, proteins, and polyesters [47].

3.2.1 Synthetic polymers

Linear or branched cationic polymers are effective transfection agents for genetic material. The structural and chemical properties of these polymers are well known. This makes them advantageous for siRNA transport. The positively charged polymers form “polyplexes” with negatively charged nucleic acid phosphates through electrostatic interactions [48].

The polymer size, the molecular weight, the degree of polymer branching, and the charge density, as well as the composition of the formulation medium and the positive and negative charges ratio between of the polymer and the oligonucle- otides, affect the transfection efficiency and biological activity of the polyplexes.

Synthetic-based cationic polymers such as PLL, PLA, and PEI are the most studied polymers for in vitro and in vivo transport of siRNA. The size of the com- plexes is one of the most important factors affecting cellular uptake. Due to their small size, the cationic polymers generally complex with the genetic material more effectively than lipids. In addition, owing to being mostly synthetic, they have some special feature such as customized size, branching, and composition, and these features can be easily changed [49].

These polymers used for siRNA delivery is well-studied, biodegradable, biocom- patible, and capable of exhibiting nucleic acid sustained release in pharmaceutical applications for decades.

PEI was used to create cationic charges on the surface of PLGA particles, which allowed the complexation of nucleic acids on the surface of the particles [50].

In a study done by Patil and Panyam [51], siRNA encapsulation studies were performed in PLGA nanoparticles. These nanoparticles were prepared by the solvent evaporation method. In this method, a cationic polymer, PEI, was added to the PLGA matrix, and ultimately it has been reported that nanoparticles can penetrate into the cell at twice the rate.

Furthermore, cationic polymers with high charge densities have “proton sponge” properties that stimulate escape from endosomes and protect genetic materials from degradation. For example, PEI, by pulling and sustaining a significant amount of protons, induces osmotic swelling and rupture of endosomes, causing the genetic material to be released from the nanoparticles in the cytoplasm and thus preventing the transport to lysosomes and degradation of genetic material [52].

In another study, the hydrogel scaffold based on polyamidoamine (PAMAM) dendrimer cross-linked with dextran aldehyde was prepared to improve the stability of the nanoparticle. These nanoparticle systems were found to be effective for gene silencing [53].

3.2.2 Natural polymers

Many polysaccharides in natural polymer structure are used for siRNA. Polysaccharides are generally biocompatible polymers. The main advantage is the presence of different functional groups (i.e., carboxyl, hydroxyl, amine) which enable functionalization to obtain structural heterogeneity and copolymers [54].

The most commonly used polysaccharides for siRNA administration include chitosan, which contains both biodegradable, biocompatible, low-cost, low cytotoxicity hydroxyl and amines. The presence of primary amino groups ($pK_a \approx 6$) makes the chitosan a polycation that promotes the association with nucleic acids and also the formation of polyplex [55].

In order to increase the solubility of chitosan, various modifications have been done and water-soluble chitoligosaccharides have been obtained. These chitoligosaccharides were used for delivery of the siRNA [35, 56].

Collagen is another biologically compatible and safe natural polymer and is a suitable carrier for drug delivery. In a study performed by Peng et al., localized and sustained release of siRNA-loaded collagen formulations were prepared for use in vivo gastric cancer, and positive results were obtained [57].

3.3 Lipid-polymer hybrid nanoparticles

Lipid-polymer hybrid nanoparticles (LPNs) were developed to eliminate the disadvantages of polymeric and lipid-based nanoparticles. The precious properties of LPNs containing polymer cores and lipid shells carry the complementary properties of both materials. In a study on the administration of LPNs in cancer treatment, the lipid/rPAA-Chol polymer hybrid nanoparticles were modified with PEG and T7 peptide; tumor has been shown to be largely inhibited without activating the immune system [58].

In another study, LPNs were used for the antitumoral effect in the pancreatic tumor model in combination with hypoxia-inducible factor 1 α (HIF1 α)-targeted siRNA and gemtubicin. This prepared LPN complex showed an excellent ability to inhibit tumor metastasis in an orthotopic tumor model [59].

4. Conclusion

In recent years, siRNA has been widely used as a promising therapeutic phenomenon to many pathological conditions. Progress has been made in researching target

genes and in the development of delivery systems for siRNA. However, challenges remain for successful clinical application of RNAi-based therapeutics. Safety concerns are the main reason for the withdrawal of clinical trials of some RNAi therapies.

Since its discovery, siRNA therapeutics have been actively used because of their high specificity, easy modifications, and unlimited therapeutic targets. However, the instability in the bloodstream and the problems with the accumulation in the target region necessitated the application of these therapeutics in a transport system.

The lipidic and polymeric nanoparticle systems described in this chapter are one step ahead the systems than other nanoparticle systems and have been proven to be of importance in these delivery processes in recent years. New modified systems are being developed to ensure safe and targeted distribution of siRNA. According to the results obtained from studies, new formulations are expected to reach clinical trials very soon such as patisiran.

Conflict of interest


The authors report no conflicts of interest. The authors alone are responsible for the content of and writing of this article.

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References

- [1] Van de Veire S, Stalmans I, Heindryckx F, Oura H, Tijeras-Raballand A, Schmidt T, et al. Pharmacological and genetic evidence for the efficacy of PlGF inhibition in cancer and eye disease. *Cell*. 2010;**141**:178-190. DOI: 10.1016/j.cell.2010.02.039
- [2] Zhao M, Sun J, Zhao Z. Synergetic regulatory networks mediated by oncogene-driven microRNAs and transcription factors in serous ovarian cancer. *Molecular BioSystems*. 2013;**9**:3187-3198. DOI: 10.1039/c3mb70172g
- [3] Fellmann C, Lowe SW. Stable RNA interference rules for silencing. *Nature Cell Biology*. 2014;**16**:10-18. DOI: 10.1038/ncb2895
- [4] Lee SJ, Kim MJ, Kwon IC, Roberts TM. Delivery strategies and potential targets for siRNA in major cancer types. *Advanced Drug Delivery Reviews*. 2015;**104**:2-15. DOI: 10.1016/j.addr.2016.05.010
- [5] Wang J, Mi P, Lin G, Wang YXJ, Liu G, Chen X. Imaging-guided delivery of RNAi for anticancer treatment. *Advanced Drug Delivery Reviews*. 2015;**104**:44-60. DOI: 10.1016/j.addr.2016.01.008
- [6] Grimm D. Small silencing RNAs: State-of-the-art. *Advanced Drug Delivery Reviews*. 2009;**61**(9):672-703. DOI: 10.1016/j.addr.2009.05.002
- [7] David S, Pitard B, Benoît JP, Passirani C. Non-viral nanosystems for systemic siRNA delivery. *Pharmacological Research*. 2010;**62**(2):100-114. DOI: 10.1016/j.phrs.2009.11.013
- [8] Giacca M, Zacchigna S. Virus-mediated gene delivery for human gene therapy. *Journal of Controlled Release*. 2012;**161**(2):377-388. DOI: 10.1016/j.jconrel.2012.04.008
- [9] Glybera: EPAR-Product Information [Internet]. 2017. Available from: https://www.ema.europa.eu/documents/product-information/glybera-epar-product-information_en.pdf [Accessed: 30 September 2018]
- [10] Jackson AL, Burchard J, Schelter J, Chau BN, Cleary M, Lim L, et al. Widespread siRNA “off-target” transcript silencing mediated by seed region sequence complementarity. *RNA*. 2006;**12**:1179-1187. DOI: 10.1261/rna.25706
- [11] FDA approves first-of-its kind targeted RNA-based therapy to treat a rare disease [Internet]. 2018. Available from: <https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm616518.htm> [Accessed: 30 September 2018]
- [12] Clinical Trials [Internet]. 2018. Available from: <https://clinicaltrials.gov> [Accessed: 30 September 2018]
- [13] Chen X, Mangala LS, Rodriguez-Aguayo C, Kong X, Lopez-Berestein G, Sood AK. RNA interference-based therapy and its delivery systems. *Cancer Metastasis Reviews*. 2018;**37**(1):107-124. DOI: 10.1007/s10555-017-9717-6
- [14] Barba AA, Cascone S, Caccavo D, Lamberti G, Chiarappa G, Abrami M, et al. Engineering approaches in siRNA delivery. *International Journal of Pharmaceutics*. 2017;**525**(2):343-358. DOI: 10.1016/j.ijpharm.2017.02.032
- [15] Vicentini FT, Borgheti-Cardoso LN, Depieri LV, de Macedo Mano D, Abelha TF, Petrilli R, et al. Delivery systems and local administration routes for therapeutic siRNA. *Pharmaceutical Research*. 2013;**30**:915-931. DOI: 10.1007/s11095-013-0971-1

- [16] Bitko V, Musiyenko A, Shulyayeva O, Barik S. Inhibition of respiratory viruses by nasally administered siRNA. *Nature Medicine*. 2005;**11**(1):50-55. DOI: 10.1038/nm1164
- [17] Wu SY, Chang HI, Burgess M, McMillan NA. Vaginal delivery of siRNA using a novel PEGylated lipoplex-entrapped alginate scaffold system. *Journal of Controlled Release*. 2011;**155**(3):418-426. DOI: 10.1016/j.jconrel.2011.02.002
- [18] Van de Water FM, Boerman OC, Wouterse AC, Peters JG, Russel FG, Masereeuw R. Intravenously administered short interfering RNA accumulates in the kidney and selectively suppresses gene function in renal proximal tubules. *Drug Metabolism and Disposition*. 2006;**34**(8):1393-1397. DOI: 10.1124/dmd.106.009555
- [19] Jain A, Jain A, Gulbake A, Hurkat P, Jain SK. Solid Tumors: A Review. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011;**3**(5):45-51
- [20] D'Apolito R, Tomaiuolo G, Taraballi F, Minardi S, Kirui D, Liu X, et al. Red blood cells affect the margination of microparticles in synthetic microcapillaries and intravital microcirculation as a function of their size and shape. *Journal of Controlled Release*. 2015;**217**:263-272. DOI: 10.1016/j.jconrel.2015.09.013
- [21] Goula D, Becker N, Lemkine GF, Normandie P, Rodrigues J, Mantero S, et al. Rapid crossing of the pulmonary endothelial barrier by polyethylenimine/DNA complexes. *Gene Therapy*. 2000;**7**(6):499-504. DOI: 10.1038/sj.gt.3301113
- [22] Wullner U, Neef I, Tur MK, Barth S. Targeted delivery of short interfering RNAs—Strategies for in vivo delivery. *Recent Patents on Anti-Cancer Drug Discovery*. 2009;**4**:1-8. DOI: 10.2174/157489209787002506
- [23] Palakurthi S, Yellepeddi VK, Kumar A. Nanocarriers for cytosolic drug and gene delivery in cancer therapy. In: Anna M, Olsztynska S, editors. *Biomedical Engineering, Trends, Research and Technologies*. Croatia: InTech; 2011. pp. 245-272. DOI: 10.5772/12918
- [24] Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. *The Biochemical Journal*. 2004;**377**:159-169. DOI: 10.1042/BJ20031253
- [25] Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. *Nature Reviews. Drug Discovery*. 2010;**9**(8):615-627. DOI: 10.1038/nrd2591
- [26] Şenel B, Büyükköroğlu G. Nanocarriers for vaccine and gene delivery application. In: Barkat A, Harshita AB, Beg S, Ahmad FJ, editors. *Multifunctional Nanocarriers for Contemporary Healthcare Applications*. USA: IGI Global; 2018. pp. 381-414. DOI: 10.4018/978-1-5225-4781-5.ch014
- [27] Santel A, Aleku M, Keil O, Endruschat J, Esche V, Fisch G, et al. A novel siRNA-lipoplex technology for RNA interference in the mouse vascular endothelium. *Gene Therapy*. 2006;**13**(16):1222-1234. DOI: 10.1038/sj.gt.3302777
- [28] Mukherjee S, Ghosh RN, Maxfield FR. Endocytosis. *Physiological Reviews*. 1997;**77**(3):759-803. DOI: 10.1152/physrev.1997.77.3.759
- [29] Sardo C, Farra R, Licciardi M, Dapas B, Scialabba C, Giammona G, et al. Development of a simple, biocompatible and cost-effective inulin-diethylenetriamine based siRNA

- delivery system. *European Journal of Pharmaceutical Sciences*. 2015;**75**:60-71. DOI: 10.1016/j.ejps.2015.03.021
- [30] Tatiparti K, Sau S, Kashaw SK, Iyer AK. siRNA delivery strategies: A comprehensive review of recent developments. *Nanomaterials (Basel)*. 2017;**7**(4):77. DOI: 10.3390/nano7040077
- [31] Wagner MJ, Mitra R, MJ MA, Baze W, Barnhart K, Wu SY, et al. Preclinical mammalian safety studies of EphARNA (DOPC nanoliposomal EphA2-targeted siRNA). *Molecular Cancer Therapeutics*. 2017;**16**(6):1114-1123. DOI: 10.1158/1535-7163.MCT-16-0541
- [32] Dominska M, Dykxhoorn DM. Breaking down the barriers: siRNA delivery and endosome escape. *Journal of Cell Science*. 2010;**123**:1183-1189. DOI: 10.1242/jcs. 066399
- [33] Chauhan H, Mohapatra S, Munt DJ, Chandratre S, Dash A. Physical-chemical characterization and formulation considerations for solid lipid nanoparticles. *AAPS PharmSciTech*. 2016;**17**(3):640-651. DOI: 10.1208/s12249015-0394-x
- [34] Botto C, Mauro N, Amore E, Martorana E, Giammona G, Bondi ML. Surfactant effect on the physicochemical characteristics of cationic solid lipid nanoparticles. *International Journal of Pharmaceutics*. 2017;**516**(1-2):334-341. DOI: 10. 1016/j.ijpharm.2016.11.052
- [35] Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. *Indian Journal of Pharmaceutical Sciences*. 2009;**71**(4):349-358. DOI:10.4103/0250-474X.57282
- [36] Şenel B, Büyükköroğlu G, Yazan Y. Solid lipid and chitosan particulate systems for delivery of siRNA. *Pharmazie*. 2015;**8**:698-705. DOI: 10.1691/ph.2015.5026
- [37] Kaur S, Nautyal U, Singh R, Singh S, Devi A. Nanostructure lipid carrier (NLC): The new generation of lipid nanoparticles. *Asian Pacific Journal of Health Science*. 2015;**2**(2):76-93
- [38] Han Y, Li Y, Zhang P, Sun J, Li X, Sun X, et al. Nanostructured lipid carriers as novel drug delivery system for lung cancer gene therapy. *Pharmaceutical Development and Technology*. 2016;**21**(3):277-281. DOI: 10.3109/10837450.2014.996900
- [39] Taratula O, Kuzmov A, Shah M, Garbuzenko OB, Minko T. Nanostructured lipid carriers as multifunctional nanomedicine platform for pulmonary co-delivery of anticancer drugs and siRNA. *Journal of Controlled Release*. 2013;**171**(3):349-357. DOI: 10.1016/j.jconrel.2013.04.018
- [40] Xue HY, Wong HL. Tailoring nanostructured solid lipid carriers for time-controlled intracellular siRNA kinetics to sustain RNAi-mediated chemosensitization. *Biomaterials*. 2011;**32**(10):2662-2672. DOI: 10.1016/j.biomaterials.2010. 12.029
- [41] Tam YYC, Chen S, Cullis PR. Advances in lipid nanoparticles for siRNA delivery. *Pharmaceutics*. 2013;**5**:498507. DOI: 10.3390/pharmaceutics5030498
- [42] Basha G, Novobrantseva TI, Rosin N, Tam YY, Hafez IM, Wong MK, et al. Influence of cationic lipid composition on gene silencing properties of lipid nanoparticle formulations of siRNA in antigen-presenting cells. *Molecular Therapy*. 2011;**19**:2186-2200. DOI: 10.1038/mt.2011.190
- [43] Basha G, Ordobadi M, Scott WR, Cottle A, Liu Y, Wang H, et al. Lipid nanoparticle delivery of siRNA to osteocytes leads to effective silencing of

SOST and inhibition of sclerostin in vivo. *Molecular Therapy--Nucleic Acids*. 2016;**5**:1-15. DOI: 10.1038/mtna.2016.68

[44] Lee JB, Zhang K, Tam YY, Tam YK, Belliveau NM, Sung VY, et al. Lipid nanoparticle siRNA systems for silencing the androgen receptor in human prostate cancer in vivo. *International Journal of Cancer*. 2012;**131**:781-790. DOI: 10.1002/ijc.27361

[45] Lin PJC, Tam YK, Cullis PR. Development and clinical applications of siRNA-encapsulated lipid nanoparticles in cancer. *Journal of Clinical Lipidology*. 2014;**9**(3):317-331. DOI: 10.2217/CLP.14.27

[46] Ball RL, Knapp CM, Whitehead KA. Lipidoid nanoparticles for siRNA delivery to the intestinal epithelium: In vitro investigations in a Caco-2 model. *PLoS ONE*. 2015;**10**:1-12. DOI: 10.1371/journal.pone.0133154

[47] Aravamudhan A, Ramos DM, Nada AA, Kumbar SG. Natural polymers: Polysaccharides and their derivatives for biomedical applications. In: Kumbar SG, Laurencin CT, Deng M, editors. *Natural and Synthetic Biomedical Polymers*. USA: Elsevier; 2014. pp. 67-89. DOI: 10.1016/B978-0-12-396983-5.00004-1

[48] Rafael D, Andrade F, Arranja A, Luís S, Videira M. Lipoplexes and polyplexes: Gene delivery applications. In: Mishra M, editor. *Encyclopedia of Biomedical Polymers and Polymeric Biomaterials*. USA: CRC Press Taylor & Francis Group; 2015. pp. 4335-4347. DOI: 10.1081/E-EBPP-120050058

[49] Tros IC, Sun Y, Düzgüneş N. Gene delivery by lipoplexes and polyplexes. *European Journal of Pharmaceutical Sciences*. 2010;**40**(3):159-170. DOI: 10.1016/j.ejps.2010.03.019

[50] Zhang XQ, Intra J, Salem AK. Comparative study of poly

(lactic-co-glycolic acid)-poly ethyleneimine-plasmid DNA microparticles prepared using double emulsion methods. *Journal of Microencapsulation*. 2008;**25**(1):1-12. DOI: 10.1080/02652040701659347

[51] Patil Y, Panyam J. Polymeric nanoparticles for siRNA delivery and gene silencing. *International Journal of Pharmaceutics*. 2009;**367**(1-2):195-203. DOI: 10.1016/j.ijpharm.2008.09.039

[52] Wang J, Lu Z, Wientjes MG, Au JLS. Delivery of siRNA Therapeutics: Barriers and Carriers. *The AAPS Journal*. 2010;**12**(4):492-503. DOI: 10.1208/s12248-010-9210-4

[53] Segovia N, Pont M, Oliva N, Ramos V, Borrós S, Artzi N. Hydrogel doped with nanoparticles for local sustained release of siRNA in breast cancer. *Advanced Healthcare Materials*. 2015;**4**:271-280. DOI: 10.1002/adhm.201400235

[54] Cavallaro G, Sardo C, Craparo EF, Porsio B, Giammona G. Polymeric nanoparticles for siRNA delivery: Production and applications. *International Journal of Pharmaceutics*. 2017;**525**(2):313-333. DOI: 10.1016/j.ijpharm.2017.04.008

[55] Ragelle H, Vandermeulen G, Pr  at V. Chitosan-based siRNA delivery systems. *Journal of Controlled Release*. 2013;**172**:207-218. DOI: 10.1016/j.jconrel.2013.08.005

[56] Mao S, Sun W, Kissel T. Chitosan-based formulations for delivery of DNA and siRNA. *Advanced Drug Delivery Reviews*. 2010;**62**(1):12-27. DOI: 10.1016/j.addr.2009.08.004

[57] Peng H, Yang H, Song L, Zhou Z, Sun J, Du Y, et al. Sustained delivery of siRNA/PEI complex from in situ forming hydrogels potently inhibits the proliferation of gastric cancer. *Journal of Experimental & Clinical*

Cancer Research. 2016;**35**(1):35-57. DOI: 10.1186/s13046-016-0334-y

[58] Gao LY, Liu XY, Chen CJ, Wang JC, Feng Q, Yu MZ, et al. Core-shell type lipid/rPAA-Chol polymer hybrid nanoparticles for in vivo siRNA delivery. *Biomaterials*. 2014;**35**(6):2066-2078. DOI: 10.1016/j.biomaterials.2013.11.046

[59] Zhao X, Li F, Li Y, Wang H, Ren H, Chen J, et al. Co-delivery of HIF1alpha siRNA and gemcitabine via biocompatible lipid-polymer hybrid nanoparticles for effective treatment of pancreatic cancer. *Biomaterials*. 2015;**46**:13-25. DOI: org/10.1016/j.biomaterials.2014.12.028

[60] Xu C, Wang J. Delivery systems for siRNA drug development in cancer therapy. *Asian Journal of Pharmaceutics*. 2015;**10**(1):1-12. DOI: 10.1016/j.ajps.2014.08.011