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Review article:

NANOCARRIERS FOR DELIVERY OF SIRNA AS GENE SILENCING MEDIATOR

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ABSTRACT

The term nanocarrier refers to sub-micrometric particles of less than 100 nm, designed to transport, distribute, and release nanotechnology-based drug delivery systems. siRNA therapy is a novel strategy that has great utility for a variety of treatments, however naked siRNA delivery has not been an effective strategy, resulting in the necessary use of nanocarriers for delivery. This review aims to highlight the versatility of carriers based on smart drug delivery systems. The nanocarriers based on nanoparticles as siRNA DDS have provided a set of very attractive advantages related to improved physicochemical properties, such as high surface-to-volume ratio, versatility to package siRNA, provide a dual function to both protect extracellular barriers that lead to elimination and overcome intracellular barriers limiting cytosolic delivery, and possible chemical modifications on the nanoparticle surface to improve stability and targeting. Lipid and polymeric nanocarriers have proven to be stable, biocompatible, and effective *in vitro*, further exploration of the development of new nanocarriers is needed to obtain safe and biocompatible tools for effective therapy.

Keywords: siRNA, nanocarrier, drug delivery systems, nanomedicine

INTRODUCTION

The term nanocarrier refers to sub-micrometric particles less than 100 nm, designed to transport, distribute, and release molecules with biological activity. These drug delivery systems are based on nanotechnology and are identified as promising strategies used to overcome technical, biological and biopharmaceutical limitations, having among their advantages, the possibility of designing multifunctional drugs with high therapeutic efficacy, thanks to the possibility of increasing specificity and selectivity for cellular or molecular targets.

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siRNA therapy is a novel strategy that has great utility in some chronic diseases, however, it has been observed that the delivery of naked siRNA has involved great difficulties, due to some of its physicochemical properties and its repercussions on biological behavior, such as its rapid degradation in biological fluids, its non-specific accumulation in tissues following systemic administration, its inability to penetrate cells by passive diffusion, and its short half-life of less than five minutes in plasma due to its susceptibility to nucleases (Sajid et al., 2020; Cullis and Hope, 2017).

The most prominent candidates for siRNA delivery are nanoparticle (NP) systems. siRNA can be incorporated into an NP formulation through covalent bonds with NP components or by electrostatic interactions with the NP surface, as acids in strongly negatively charged nuclei tend to form complexes. In addition, NP has been considered as specific and safe nanocarriers since they offer a set of advantages such as a high surface-to-volume ratio, a significant increase in bioavailability and a decrease in clearance of low bioavailable active ingredients (APIs), as well as their ability to preferentially accumulate on a selected target (see Figure 1) (Mainini and Eccles, 2020).

Nanocarriers based on nanoparticle formulations allow organ-specific targeting and provide a wide versatility to package siRNA with multifunctional performance due to their surface modifications, thus enabling the delivery of macromolecules via cellular and even transcellular pathways. In addition, it is suggested that nanoparticle systems can promote endosomal escape by different pathways such as the "proton sponge effect", membrane fusion, membrane destabilization or induced swelling, thus preventing late endosome elimination in conjunction with API, which is very useful for siRNA delivery, since it enters cells by endocytosis like most nanoparticles (Lin et al., 2020; Ashrafizadeh et al., 2020; Kim et al., 2019a; Chevalier, 2019; Smith et al., 2019; Singh et al., 2018).

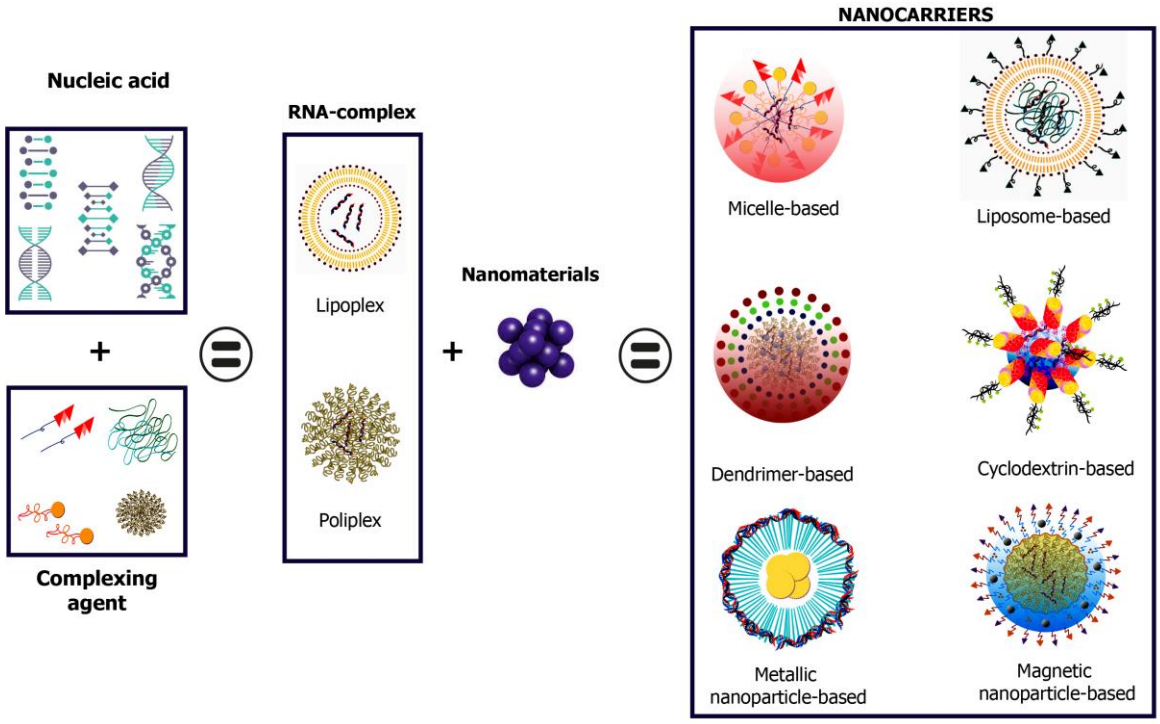

Figure 1: siRNA delivery systems can be constructed from a variety of materials with varying physicochemical features and biological behavior.

Interest in the development of siRNA nanocarriers began with gene therapy through the transfer of nucleic acids, including siRNA, microRNA (miRNA), short hairpin RNA (shRNA), antisense oligonucleotides (ASOs), aptamers, mRNA, plasmid DNA (pDNA), and CRISPR-Cas9. Exponential growth in the areas of molecular biology, pharmaceutical technology and materials science has enabled the design of effective pharmaceutical formulations that are currently in clinical trials and commercialization.

siRNA delivery systems can be constructed from a variety of materials with varying characteristics (Figure 1), all of which contribute to maximizing therapeutic potential. For this review, we will present and classify only systems composed of lipidic, polymeric, and inorganic nanoparticles, which make up micelles, liposomes, polymer blocks, hydrogels, etc., each with different physicochemical properties that allow for specific siRNA charge depending on the type of nanoparticle (Sharma et al., 2020; Chenthamara et al., 2019).

Small interfering RNA (siRNA) is a non-coding RNA-type oligonucleotide (ncRNA; ~2 nm and ~13.5 kDa), its role in mediating post-transcriptional gene silencing has been widely studied and it has been established that binding to the RNA-Induced Silencing Complex (RISC, multi-protein complex) guides the specific degradation of messenger RNA (mRNA) preventing its translation into a protein. There are six siRNA drugs in late stages of Phase 3 clinical trials, including *vutrisiran, nedosiran, fitusiran, teprasiran, cosdosiran*, and *tivanisiran*. The use of siRNA in recent decades has become a promising therapeutic alternative to address gene overexpression for various pathological conditions, providing significant advantages regarding pharmacological inhibitors, highlighting its specific binding activity; meaning that siRNA can selectively bind to a target mRNA allowing the silencing of desired genes (Kokkinos et al., 2020; van den Brand et al., 2018; Sarkies and Miska, 2014; Lin et al., 2020).

In summary, there are 16 approved nucleic acid drugs: 9 ASO-based, 4 siRNA-based, 1 aptamer-based, and 2 mRNA-based (the latter being *Tozinameran* developed by Pfizer/BioNTech and *Elasomeran* developed by Moderna, which were designed for the prevention of coronavirus-19 (COVID19), they were approved at the same time in 2020) (Paunovska et al., 2022; Zhuang and Cui 2021; Hodgson, 2021; Ferenchak et al., 2021).

NANOCARRIERS COMPOSED OF LIPID NANOPARTICLES

Lipids have the natural tendency to enhance cellular uptake of siRNA, with the added advantage of being very simple to formulate, have great versatility in their function, with diverse and programmable release profiles, just by modifying their lipid matrix and functionalizing molecules. Lipid nanocarriers are generally biodegradable, biocompatible, non-immunogenic or low immunogenic and have tolerable or low toxicity. However, in some cases, these nanocarriers are not completely inert, because some cationic lipids (amphiphiles with quaternary ammonium head groups) can reduce mitosis in cells, form vacuoles in the cytoplasm, and cause detrimental effects on key cellular proteins such as protein kinase C. On the other hand, notable disadvantages include their limited stability, their relatively low capacity to load siRNA, and, occasionally, the possible interaction and breakdown of payloaded nucleic acids (Han et al., 2021; Inglut et al., 2020; Scheideler et al., 2020; Zatsepin et al., 2016; Tenchov et al., 2021).

RNA lipid nanocarriers are called lipoplex, which refers to systems composed of a combination of cationic lipids with nucleic acids, their formation consists of two steps, first, the cationic lipidic environment promotes electrostatic interactions, while the second step concerns the rearrangement and condensation of the lipoplex, forming structured self-assembly between lipids and phosphate groups from the siRNA main chain. The ge-

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ometry of lipids determines the phase structure (micellar, lamellar, cubic, and inverted hexagonal phase) according to the packing parameter, moreover, it is well known that the phase plays a significant role in physicochemical and biological behavior, determines digestibility, absorption pathway, distribution, uptake, and delivery mechanisms (see Figure 2). The most common lipid nanocarriers for siRNA delivery are micelles, liposomes, and lipid solids, but other lipid formulations are currently being studied and will later be discussed (Berger et al., 2021; Fairman et al., 2021; Kokkinos et al., 2020).

Micelles

Micelles are amphiphilic systems of small lipid vesicles with spherical shape, they have a hydrophobic core and a hydrophilic shell, they are produced by spontaneous self-assembly in aqueous media, their formation depends on amphiphile concentration, temperature, solvent, and size of hydrophobic/ hydrophilic domains, they can protect RNA/DNA and/or drugs in their micellar core, due to their small size (≤ 100 nm), they are applied for siRNA release, in most cases, they are conjugated with polymers to avoid binding to negatively charged serum proteins, also to prevent their aggregation, and to provide steric stabilization (Ojo et al., 2021).

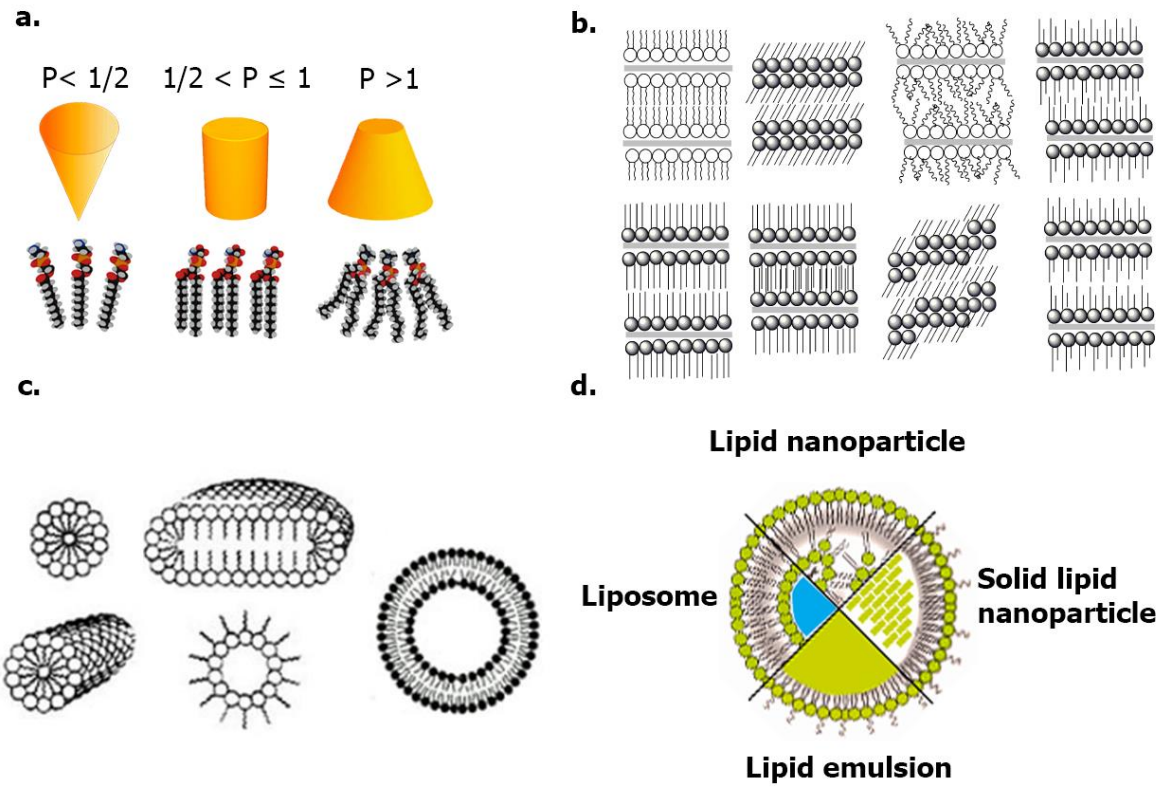

Figure 2: a. Lipid packing parameters and phases (micellar, bilayer, hexagonal); **b.** Varieties of lipid phases (lamellar, sub-gel, gel, liquid crystalline, etc.); **c**. Lipid self-assembly aggregates (Koynova and Caffrey, 1998); d. Lipid nanocarriers for delivery

Liposomes

Liposomes are large, closed spherical vesicles constructed from a lipid bilayer, which could be classified according to their size (~0.025–5 μm) and their number of lipid bilayers (unilamelar or multilamellar vesicles), composed of different types of phospholipids, cholesterol and steroids bounding the hydrophilic core. Being formed by the self-assembly of amphiphilic molecules, the components are arranged so that they can be used as nanocarriers for both hydrophobic and hydrophilic components. These systems have a high degree of biocompatibility, degradability, efficacy, encapsulation capacity for plenty of APIs and ease of formulation. Liposomes serve as smart release systems in conjunction with various functionalizing agents, so they have been the standard for siRNA transfection (Ajeeshkumar et al., 2021; Aldosari et al., 2021; Majumder and Minko, 2021; Charbe et al., 2020; Bholakant et al., 2020).

Solid lipids nanoparticles

Other lipid nanocarriers are those composed of solid lipid nanoparticles (SLNs) with a size of around 100-200 nm, which are micellar vesicles formed by colloidal nanoparticles grouped in a lipid monolayer enclosing a solid, hydrophobic lipid core; they are formed after emulsion with a surfactant that stabilizes the lipid dispersion, their function is to prevent permeation and degradation of their components, they have the advantage of being highly biocompatible, moreover, they have good storage stability and provide the opportunity to carry out a sterilization and lyophilizing process if required, therefore, this type of nanocarrier can incorporate lipophilic or hydrophilic molecules such as siRNA following several strategies (Basha et al., 2021; Dhiman et al., 2021; Khalid et al., 2020; Yonezawa et al., 2020; Scheideler et al., 2020).

Miscellaneous lipid nanoparticles for siRNA delivery

The most commonly used lipid nanocarriers are liposomes, solid lipid NPs and nanostructured lipid carriers, among others, all of which have long-term physicochemical stability as nano-emulsions. Table 1 summarizes the reported lipid nanocarriers of nucleic acids. The droplet size range is 55 to 209 nm, with toxicity of less than 30 %, and *in vitro* gene knockdown ranging from 50 to 98 %. These nanocarriers are mainly designed for breast cancer therapy, and the predominant routes of administration are parenteral (intravenous (IV) or intratumoral (ITI) injection). Hybrid systems have been reported, composed with other types of nanoparticles such as polymeric ones, mainly forming liposomes that promote structural modifications with PEG to increase stability in plasma and avoid non-selective adhesion or, similarly, lipid nanocarriers with surface modifications with peptides, proteins, antibodies or aptamers (Herceptin or hyaluronic acid) that act as ligands to direct the nanocarriers to specific targets are observed (Rehman et al., 2020; Scheideler et al., 2020). The nanocarrier proposed by Ball et al. (2018) provides an example of a lipid nanocarrier designed for oral administration of siRNA, composed of a mixture of lipoid 3060, cholesterol, DSPC, and PEG 200-DMG with a size of about 140 nm, PDI of 0.12, and a ζ potential of ± 10 mV. Unfortunately, this nanocarrier did not effectively knockdown GAPDH *in vitro* and *in vivo* in Caco-2 cells and in the mouse model respectively, demonstrating that gene silencing efficiency may be affected mainly by pepsin, bile salts, and mucin concentrations, when the nanocarriers are administered orally since the nanocarriers are destabilized, altered, and trapped in the gastrointestinal (GI) tract environment.

Table 1: Nanocarriers composed of lipid nanoparticles

Nanocarrier/ goal	Route[a]	Size (nm)	ζ (mV)	Nanocarrier toxicity	Gene silencing	Reference
Solid lipid nanoparticle / papilloma	ITI	140.4 ± 12.9	43.9 ± 2.7	KB cells: no data	MCL1: ~ 60% *in vitro*	Yu et al., 2012
Components: retinol, DC-Chol, DPhPE, EDOPC, mPEG-DSPE, glyceryl trioleate, and PTX						
Nanosphere / Myeloid Leukemia	IV	55	No data	K562 cells: < 30%	BCR-ABL: 90% *in vitro*	Jyotsana et al., 2019
Components: ionizable cationic lipid and PEG-DMG						
Micelle / colon cancer	ITI	144.8	46.4	C26 cells: <10%	Bcl.xl: ~75% Mcl-: ~50% *in vitro*	Lu et al., 2019
Components: DOTAP and mPEG-PCL						
Liposome / cervical cancer	ITI	~200	No data	HeLa: non-toxic (0%)	Luc: 95% *in vitro*	Xu et al., 2013
Components: G0-C14 (cationic lipid), PLGA-PEG, and cisplatin						
Liposome / lung cancer	IV	165.4 ± 1.7	~13	H226 and A549 cells: <10%	Tumor inhibiting rate: ~75% *in vivo*	Qu et al., 2014
Components: DPPC, DSPE-PEG, DDAB, and DTX						
Liposome / ovarian cancer	ITI	156.3 ± 6.7	-3.1 ± 0.5	SKOV-3 cells: ~5%	Bcl-2: ~85% P-gp: ~ 70% *in vitro*	He et al., 2015
Components: DOTAP, DOPA, cholesterol, DSPE-PEG, and cisplatin						
Liposome / lung cancer	IV	~102 ±. 2.6	22.5 ± 3.6	NCI-H460 cells: no data	Luc: 70%–80% *in vitro*	Shi et al., 2017
Components: DSPE-mPEG-AA, Metformin-cholesterol, DOPE, HA, and protamine						
Liposome / cervical cancer	PR	6.1 ± 0.3 (MLM)[b]	~ 20	HeLa cells: 10-20%	GFP: ~ 66 % *in vitro*	Sánchez-Arribas et al., 2020
Components: C3(C16His)2 (Gemini Cationic Lipid) and MOG						
Liposome / breast cancer	IV	~ 181.3	~ 36.6	MCF-7 cells: ≤ 20%	Luc: ~ 98% *in vitro*	Hattori et al., 2020
	IV	209	~ 32.7	MCF-7 cells: 10 – 25%	Luc: ~ 95% *in vitro*	
	IV	181	~ 27.4	MCF-7 cells: < 20%	Luc: ≥ 90% in vitro	
Components: DOPE, DDAB, CS, and PEG (1) / DOPE, DOTAP, CS, and PEG (2) / DOPE, TC-1-12, CS, and PEG (3), respectively						
Liposome / breast cancer	PR	176 ± 54	28.1 ± 1.8	SK-BR-3 cells: ~20%	CDK4: 62-68% *in vitro*	Shin et al., 2020
Components: DOPE, DC-Chol, HA, protamine, and PEG- thiolated Herceptin						
Liposome / breast cancer and colon cancer	PR	92.4 ± 24.5	-33.6 ± 4.5	MCF-7 and HT-29 cells: < 20%	C-myc (MCF-7): ~ 95% *in vitro* C-myc (HT-29): ~ 90% *in vitro*	Habib et al., 2021
	PR	126.8 ± 7.3	-43.9 ± 5.4	MCF-7 and HT-29 cells: < 15%		
Components: MS09 and DOPE (1) / MS09 and Chol (2), respectively						
Liposome / lung cancer	PR	~ 170	~ 15	A549 cells: < 10%	PD-L1: ~70% *in vitro*	Gao et al., 2021b
Components: DOPC, Chol, and PEI- stearic acid						

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Table 1 (cont.): Nanocarriers composed of lipid nanoparticles

Nanocarrier/ goal	Route[a]	Size (nm)	ζ (mV)	Nanocarrier toxicity	Gene silencing	Reference
Components: DOPC, Chol, and PEI- stearic acid						
Liposome / cancer	PR	122 ± 16	5.9 ± 0.9	EA.hy 926 cells: non-toxic	GFP: ~55% *in vitro*	Ahmed et al., 2021b
	PR	143 ± 12	8.9 ± 1.8 mV	EA.hy 926 cells: no data	GFP: ~50% in vitro	
Components: DOPC, poloxamer (P407) and DMAPAP (PvcLDMAPAP) (1) / DOPC, poloxamer (P407) and PEI (PvcLPEI) (2), respectively						

(a) ITI: Intratumoral injection, IV: Intravenous injection; b: Multilamellar, PR: parenteral route

POLYMER NANOPARTICLE-BASED NANOCARRIERS

Polymer-based nanocarriers represent the second most widely used carrier type for siRNA delivery and are more robust and stable nanocarriers than lipid nanocarriers, generally referred to as polyplex, which is a complex between the cationic groups of the polymer and the phosphate group of the nucleic acid. Polymeric nanoparticles can be used to protect siRNA by modifying its ionizable groups or by varying its size, consequently, siRNA increases its absorption rate and they can be classified as vesicular systems (nanocapsules) and matrix systems (nanospheres) (Castro et al., 2022; Kim et al., 2021). In the particular case of siRNA, polymeric systems condense or form a complex with siRNA, as a consequence, these nanocarriers can be found in the form of micelles, polymersomes, dendrimers or cyclodextrin polymers (Witika et al., 2020; Vasile, 2019; Castro and Kumar, 2013).

These nanocarriers are mainly composed of cationic or ionizable polymers (see Table 2), to protect the siRNA payload and increase its cellular uptake, thus finding systems with linear, branched and/or block copolymers that have the ability to bind siRNA through covalent bonds (linear and branched copolymers) or bind through their amphiphilic properties to encapsulate siRNA (block copolymers) (Patel et al., 2021; Ahmed et al., 2021a; Itani and Al Faraj, 2019).

Polymeric micelles

Polymeric micelles are supramolecular self-assemblies with different morphologies (spheres, discs, and worm-shaped assemblies), composed of amphiphilic synthetic macromolecules in which the individual block copolymers are generally linked by non-covalent interactions, solubilizing the API in their core, while their shell allows them to be suspended in the aqueous medium. Polymeric micelles are considered a good system siRNA delivery because they use the core-shell structure for delivery and are smaller in size (< 200 nm) and more efficient for cellular internalization than other polymeric nanocarriers, they also have a high loading efficiency, are versatile, stable under physiological conditions and can be divided into two categories: (1) micelles formed from direct binding of polymers via covalent (non-degradable) bonds to siRNA and (2) micelles formed from direct condensation of siRNA with amphiphilic polymer block (Wan et al., 2021; Ghezzi et al., 2021; Charbe et al., 2020).

Polymersomes

Polymersomes are characterized as spherical cavitary bodies with a bilayer membrane between 2-47 nm in size, morphologically similar to lipid-based vesicles but consisting of amphiphilic block copolymers. These nanocarriers show a lower permeability to water and can tolerate much more areal pressure before rupture. Consequently, they

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Table 2: Nanocarriers composed of polymeric nanoparticles

Nanocarrier/ Treatment	Route	Size (nm)	ζ (mV)	Nanocarrier toxicity	Gene silencing	Reference
Polymersome / lung cancer	PR	100	No data	A549 cells: non-toxic	Lamin A/C: ~40% *in vitro*	Kim et al., 2009
Components: PEG-polylactic acid (OLA)						
Polymersome / breast cancer	PR	~ 250	No data	MCF10A cells: non-toxic	Orai3: ~85% *in vitro*	Pangburn et al., 2012
Components: OB, PR_b and GRGDSP						
Polymersome / stomach cancer	No data	168.9 ± 8.3	No data	MKN-45 and MKN28 cells: no data	Bcl-xL: 68-80% *in vitro*	Kim et al., 2013
Components: mPEG-b-PLA and DOX						
Polymersome / cancer	ITI	~227	- 40 to - 60	293T cells: no data	GFP: ~80% *in vitro*	Noh et al., 2011
Components: α-tocopherol oligochitosan (TCOsome4K)						
Polymersome / hepatic cancer	PR	232	11	L02 cells: non-toxic	miR-429 (HCCLM3): ~80% *in vitro*	Chen et al., 2015
Components: PEO-b-PDPA-b-PAA, Antibody, EpCAM, Streptavidin, and DOX-HCl						
Polymersome / sundry cancers	PR	173 ± 7	Neutral	B16F10, MCF-7 and KB cells: <20%	Luc (B16F10): 31% *in vitro*	Gallon et al., 2015
Components: $mPEG_{43}$-pImHeMA$_{67}$-pGMA$_{36}$, tBocPEG$_{80}$-pImHeMA$_{20}$-pGMA$_{58}$, and folate-PEG$_{80}$-pIm-HeMA$_{20}$-pGMA$_{58}$						
Polymersome / hepatic cancer	PR	203	31.9	HepG2 cells: no data	No data	Li et al., 2015
Components: PAsp(DIP)-b-Plys and DOX (N/P 2)						
Polymersome / lung cancer	IV	101-175	Neutral	A549 cells: non-toxic	PLK1: ~ 75% *in vitro*	Zou et al., 2017
Components: PEG-P(TMC-DTC)-PEI and cNGQ-PEG-P(TMC-DTC) (400 nM siRNA)						
Polymersome / cancer	SC	462	~30	L02 cells: ~10%	FAM: no data	Wang et al., 2018
Components: PEO-b-P(NIPAM-stat-CMA-stat-DEA) (37°C and 40 µg /mL)						
Polymersome / breast cancer	No data	131.5-137.7	No data	MCF-7cells: ~5%	P-gp: ~60% *in vitro*	Zheng et al., 2019
Components: PNIPAM orthopyridyl disulfide, mercapto siRNA and DOX-HCl						
Polymersome / lung cancer	IV	90	Neutral	A549 cells: no data	PLK1: ~85% *in vitro*	Qiu et al., 2019
Components: PEG-b-PAPA-b-PLL and CPP33-PEG-bPAPA (400 nM siRNA)						

IV: Intravenous injection, PR: parenteral route, SC: subcutaneous injection, ITI: intratumorally injection

are resistant, stable and are used to administer both hydrophilic and hydrophobic APIs, however, their slow release may sometimes be a disadvantage due to their membrane thickness. On the other hand, thanks to the characteristic self-assembly of amphiphilic block copolymers, they can maintain their well-defined structure in an aqueous media promoted by a thermodynamic phenomenon between non-covalent physical interactions. Due to the physicochemical versatility of polymersomes, their increased stability and improved payload retention, they are used for the delivery of nucleic acids and/or macromolecules for both *in vitro* and *in vivo* delivery (pDNA, AON, siRNA) (Scheerstra et al., 2022; Araste et al., 2021; Moulahoum et al., 2021; Iqbal et al., 2020).

Dendrimers

Dendrimers are a class of highly stable spherical nanoparticles with high biocompatibility and resistance to proteolytic digestion, macromolecules characterized by their symmetry and 3-D globular architecture, consisting of a central core, inner branches, and outer surface. Dendrimers have a well-defined shape, a highly monodisperse size, and a chemical homogeneity resulting from their repetitive branched pattern. In addition, they have significant advantages over linear polymers in that they have a higher loading capacity, a larger number of high-density surface functionalities that allow them to conjugate with other components. These nanocarriers called dendriplex, easily encapsulate siRNA and are optimal for delivery because their protonated amines induce an endosomal osmotic burst resulting in cytoplasmic accumulation of siRNA (Pishavar et al., 2021; Subhan et al., 2021; Yan et al., 2021; Bholakant et al., 2020).

Cyclodextrin polymers

Cyclodextrins (CD) are crystalline, homogeneous, non-hygroscopic substances with different sizes, belonging to the family of tricyclic oligosaccharides composed of glucopyranose units, they are differentiated according to their number of units; αCD (6), βCD (7), and γCD (8), they have been used as excellent solubilizers and stabilizers thanks to their torus-like macro ring shape and their relatively hydrophobic cavity associated with an aqueous environment that allows them to form "host-guest" inclusion complexes, where the dissolved CD (host) allows energetically disadvantaged water molecules to move into their cavities with the "guest" molecule (ions, proteins, or oligonucleotides). Cyclodextrin polymers can be defined as molecules containing more than two covalently linked CD units, they are used to provide an alternative to conveniently deliver hydrophobic/hydrophilic molecules, thus, these systems are nanocarriers that could provide safe, effective, and targeted delivery of siRNA (Xu et al., 2021a; Pandey et al., 2022; Pandey, 2021; Mousazadeh et al., 2021; Petitjean et al., 2021; Liu et al., 2021b; Yao et al., 2019; Ceborska, 2017).

Miscellaneous polymeric nanocarriers for siRNA delivery

Some polymeric nanocarriers are shown in Table 3, where the outstanding use of polymers such as PEG, PCL, PEI, and PNIPAM is observed. At the same time, it is observed that polymeric nanocarriers are conjugated with ligands, such as peptides, folic acid, and hyaluronic acid, and even hybrid polymeric nanocarriers composed of inorganic nanoparticles are observed. In general, these nanocarriers have sizes ranging from 7 to 591 nm, toxicity of less than 50 %, and gene knockdown ranging from 20 and 90 % *in vitro*, and are mainly used for melanoma, administered by IV injection. Additionally, other nanocarriers designed for the oral administration of siRNA, proposed by He et al. (2020) are presented, these nanocarriers were composed of mannose-modified trimethyl chitosan-cysteine (MTC) and anionic cross linkers including TPP, HA, and Eudragit® S100, their properties were a size range between 120 and 225 nm and a ζ potential of 18-37 mV, they also

showed an effective *in vitro* TNF-α knock-down of 25-75 % in Raw 264.7 cells and no significant toxicity (<10 %). These results in simulated gastric fluid are due to mucoadhesive properties of the three functional groups (trimethyl, thiol, and mannose) of the nanocarrier that promote oral absorption and the use of Eudragit® S100 that does not dissolve the system down to a specific pH.

Table 3: Nanocarriers composed of miscellaneous polymeric nanoparticles

Nanocarrier/ Treatment	Route	Size (nm)	ζ (mV)	Toxicity	Gene silencing	Reference
Micelle / melanoma	PR	132.2 ± 2.6	29.3 ± 1.2	MDA-MB-435 cells: 20%	VEGF:85% *in vitro*	Zhu et al., 2010
Components: PDMAEMA–PCL–PDMAEMA and PTX						
Micelle / melanoma	IV	103.4 ± 5.1	4.23 ± 0.51	MDA-MB-435 cells: no data	MDR1: no data	Xiong and Lavasanifar, 2011
Components: Acetal-PEO-b-PCL, polyamine, TAT, RGD, and DOX						
Micelle / hepatic cancer	PR	~190	18.9	Bel-7402 cells: <20%	Bcl-2: >50% *in vitro*	Cao et al., 2011
Components: PEI-PCL, FA-PEG-PGA, and DOX						
Micelle / melanoma	IV	50	No data	MDA-MB-435 cells: no data	Plk1: 32% - 78% *in vitro*	Sun et al., 2011
Components: mPEG-b-PCL-b-PPEEA and PTX						
Micelle / breast cancer	IV	121.3 ± 1.9	20.48 ± 1.8	MCF-7 cells: <10%	Bcl-2: 32% -78 % *in vitro*	Zheng et al., 2013
Components: PEG-PLL-PLLeu and DTX						
Micelle / breast cancer	PR	243 ± 12.1	36.33 ± 4.5	MCF-7/ADR: no data	P-gp: >75% *in vitro*	Misra et al., 2014
Components: PLGA, DMAB, PVA, TPGS, and DOX						
Micelle / lung cancer	IV	43	neutral	A549 cells: no data	GFP: ~45% *in vitro*	Zhu et al., 2014
Components: PEG-pp-PEI-PE and PTX						
Micelle / ovarian cancer	IV	64	5.3	SKOV-3 cells: <30%	Bcl-2: ~ 60 % *in vitro*	Chen et al., 2014
Components: PEG-PAsp(AED)-PDPA and DOX						
Micelle / breast cancer	IV	80-140	16-36	4T1 cells: 27.1%	Tumor inhibiting rate: 76.5% *in vivo*	Tang et al., 2015
Components: PEI-PDHA, PEG-PDHA, and PTX						
Micelle / ovarian cancer	IV	~25	No data	SKOV3-tr cells: non-toxic (0%)	Survivin: 90 % *in vitro*	Salzano et al., 2015
Components: PEG2000-PE and PTX						
Micelle / hepatic cancer	ITI	~200	20	SMMC-7721 cells: ~25%	VEGFA: ~50% PGL3: ~75% *in vitro*	Yuan et al., 2020
Components: PEI with heptafluorobutyric anhydride						
Micelle / hypopharyngeal carcinoma	PR	120	~6	FaDu cells: ~10%	Luc: 40-50% *in vitro*	Fliervoet et al., 2020
Components: PNIPAM-PEG-pDMAEMA and PNIPAM-PEG-PNIPAM (N/P5/37°C/500 nM)						
Micelle / asthma	IN	150 – 275	2.5 - 7.5	16-HBE cells: ~20%	IL-4: <45% *in vitro*	Craparo et al., 2020
Components: PHEA-g-PEG-g-bAPAE (35 % DD bAPAE) / (p/p :10)						
Micelle / melanoma	IV	51.2 ± 1.3	5 ± 0.5	B16-F10 cells: <20%	RelA: >50% *in vivo*	Ibaraki et al., 2020
Components: mPEG-PCL and functional peptide ($CH_2R_4H_2C$) / (N/P 10)						

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Table 3 (cont.): Nanocarriers composed of miscellaneous polymeric nanoparticles

Nanocarrier/ Treatment	Route	Size (nm)	ζ (mV)	Toxicity	Gene silencing	Reference
Micelle / melanoma	IV	326.1 ± 35.0	-13	MDA-MB-231 cells: <20%	PLK1: ~80 % in vitro PLK1: 90% in vivo	Li et al., 2021a
Components: 4-MAPBA-NIPAM-Bis-Acridita-DNA (DPNF) and ATP						
Dendrimer-based / glioblastoma	PR	No data	No data	U87MG cells: non-toxic until 5.5 µg/mL	Luc: 75% in vitro	Kaneshiro and Lu, 2009
Components: poly(L-lysine) G3, PEG-RGD, and DOX						
Dendrimer-based / ovarian cancer	ITI	85	32	SKOV-3 cells: non-toxic (0%)	Akt:50% *in vitro*	Kala et al., 2014
Components: PANAM G6 and PTX						
Dendrimer-based / ovarian cancer	PR	175.8 ± 1.04	4.55 ± 0.25	A2780 ADR cells: <25%	P-gp: 40 % *in vitro*	Pan et al., 2019
Components: PANAM G4-PEG2000-DOPE, PEG-DOPE, and DOX						
Dendrimer based / glioblastoma	IV	200	neutral	U87MG cells: ~30%	VEGFA: ~25% *in vitro*	Bai et al., 2020
Components: Oligo-spermine-imidazole-diimine, PANAM G6, and tri-block copolymer						
Dendrimer-based / prostatic cancer	IV	591.1 ± 6.6	-1.40 ± 0.14	PC-3 cells: Non-toxic (0%)	Luc: ~80 % *in vitro*	Noske et al., 2020
Components: Tyrosine-Modified PPI (PPI-G4-Y)						
Cyclodextrin polymers -based / papilloma	IV	~150	~7.5	KB cells: nontoxic (0%)	Bcl-2: 20% GFP: 88% *in vitro*	Wen et al., 2020
Components: β-CD-SS-pDMAEMA and Ad-PEG-FA						
Nanocapsule / papilloma	ITI	~7	neutral	KB cells: <50%	EG5 (KSP): ~ 80 % *in vitro*	Lee et al., 2016
Components: Succinoyl tetraethylene pentaamine, α-amino acids, PEG, MTX, and Inf7 peptide						
Nanocapsule / glioblastoma	IV	25.6	neutral	U87MG Cells: non-toxic (0%)	PLK1: 66 % *in vitro*	Zou et al., 2020
Components: Acrylate guanidine, N,N′-bis(acryloyl) cystamine, PEG and Angiopep-2						

ITI: Intratumoral injection, IV: Intravenous injection, IN: Intranasal, PR: parenteral route

NANOCARRIERS COMPOSED OF INORGANIC NANOPARTICLES

Inorganic nanoparticles (INPs) for siRNA delivery are generally composed of different types such as metallic nanoparticles, where gold nanoparticles stand out, super-magnetic nanoparticles, mainly iron oxides, semiconductor nanoparticles such as quantum dots, and ceramic nanoparticles, mainly mesoporous silica. INPs have emerged as valuable

building blocks with continuous breakthroughs, particularly in their optical, electronic, magnetic, and catalytic properties, making them capable of detecting, diagnosing, and treating many diseases, and thus have numerous biomedical applications, including siRNA therapy. Moreover, INPs are synthesized through a variety of methods, creating extremely organized three-dimensional structures which can be modified with ligands to improve their affinity, they also have fairly attractive advantages such as precise control of nanocarrier size, high loading efficiency, control of API release, tunable surface properties, inertia, high stability, good reproducibility, easy cellular absorption, long useful life, and very attractive physical properties, making them prominent as theragnostic agents and recently as functional nanocarriers for siRNA and chemotherapeutic agents (Lins et al., 2021; Torres-Vanegas et al., 2021; Khan et al., 2021; Khalid et al., 2020; Khurana et al., 2019).

For effective siRNA delivery, it is essential that these nanocarriers have external functionalization. Thus, INPs can form a coordination network between siRNA and organic nanoparticles, which generally increase their efficiency by improving their biocompatibility and protecting them from oxidation. In addition, INPs can be anchored to siRNA by physical adsorption, covalent coupling, or metal-ligand interactions. This versatility in incorporating siRNA has caused some of these nanocarriers to reach the advanced stage for clinical development, although most of them are still in the early stages (Zou et al., 2021; Yau et al., 2021; Jiang and Thayumanavan, 2020; Charbe et al., 2020).

Metal-based nanoparticles: AuNPs

Among the nanocarriers composed of metallic nanoparticles, gold nanoparticles (AuNP) are commonly used since they have unique biochemical properties and can be created with a wide versatility of shape, size (~15-50 nm using the Turkevich method), and tunable surface charges, they also have good properties such as non-toxicity, biocompatibility and can be easily adsorbed to the surface of APIs or can bind through covalent thiol bonds. In addition, these nanocarriers can induce a controlled release through different strategies and offer unique optical and electronic properties due to their strong localized surface plasmon resonance (LSPR). Gold nanoparticles coated with polymers or conjugated to another molecular compound have been extensively studied as siRNA delivery systems, since they have successfully demonstrated to be effective in gene knockdown, have no detectable off-target effects, and also provide a photothermal therapeutic effect as a secondary function, making them even more attractive as nanocarriers (Pylaev et al., 2021; Aghamiri et al., 2021; Moore and Chow, 2021; Gumala and Sutriyo, 2021).

Base-magnetic nanoparticles: SPIOs

Magnetic nanoparticles (MNPs) are a new type of magnetic nanocrystals composed of iron, nickel, cobalt, or magnesium. Iron oxides (Fe_3O_4 or Fe_2O_3) are the most important MNPs because they can produce strong paramagnetism, even superparamagnetism (SPIO, iron oxides with a diameter <50 nm) and are also safer than cobalt or nickel, which are reported to be more toxic. SPIOs possess advantages such as uniform size, large surface areas, high surface-to-volume ratio, a rapid transfection process and efficient biodegradability. In addition, SPIOs can provide target-oriented delivery because they interact with external magnetic fields (EMF) that allow them to be lead to target sites, even to hard-to-transfect and non-permissive cells, at the same time, they can provide molecular imaging and a magnetocaloric effect which can indirectly kill tumor cells, which is why these nanocarriers are used for siRNA delivery. These nanocarriers are highly efficient for releasing siRNA, thanks to magnetofection, a technique to enhance the efficiency of transfection of nucleic acid with EMF, but require improvements in their colloidal stability, so they usually have surface modifications using polymeric cross linkers that encapsulate these

nanoparticles (Li et al., 2021b; Bassetto et al., 2021; Maurer et al., 2021; Liu et al., 2021a; Huang et al., 2021; Dowaidar et al., 2017).

Semiconductor-based nanoparticles: QDs

Quantum dots (QDs) are colloidal semiconductor nanocrystals with sizes <10 nm, in general, their structure consists of a core and shell, composed of group II-VI elements (CdTe, ZnS, and CdSe), group III-IV elements (InAs and InP) or group III-IV elements IV-VI (CS, CSe, PbS, and PbSe). They can be classified into three main types: (1) according to their composition/structure: core-type (formed with a single component), core-shell type (core encapsulated by a semiconducting substance) and alloyed (formed with two semiconducting materials), (2) according to the material used for their preparation: semiconducting QDs and carbon/graphene QDs and (3) according to their size; large (5-6 nm) and small (2-3 nm). QDs have optical properties, absorbance and photoluminescence dependently allowing *real-time in situ* monitoring delivery of APIs. Additionally, they have long-term stability, wide-field excitation, an extensive emission spectrum and non-toxic effects, making them great candidates for theranostic therapy in conjunction with siRNA, however, these nanocarriers need superficial alterations employing hydrogel or covalent interlayer bonding to explicitly bind with nucleic acids (Singh et al., 2021; Gidwani et al., 2021; Tandale et al., 2021; Khalid et al., 2020; Kim et al., 2017).

Ceramic-based nanoparticles: MSNs

Ceramic nanoparticles are a relatively new type of porous inorganic nanoparticles for siRNA delivery, composed of silica, titanium oxide, calcium phosphate and alumina. These ceramic nanoparticles provide a tunable nanocarrier in both pore diameter (2-50 nm) and pore volume (> 0.9 cm^3/g), as well as surface functionalization capability, high surface area, good biocompatibility, degradability, high loading capacity and chemical inertness. Mesoporous silica nanoparticles (MSNs) are the most relevant ceramic nanoparticles for siRNA delivery, having hundreds of empty channels that assemble into two- or three-dimensional porous structures, where they can load APIs such as siRNA. On the other hand, they are protonated by amination or coating with cationic polymers to enable electrostatic interactions with siRNA. Moreover, they can be functionalized with ‘‘molecular gates” and have external stimuli to allow charge delivery to be triggered (Gao et al., 2021a; Yau et al., 2021; García-Fernández et al., 2021; Taleghani et al., 2021; Lins et al., 2021).

Miscellaneous inorganic nanocarriers for siRNA delivery

To conclude this classification, Table 4 shows some examples of inorganic nanocarriers, where it is observed that Au-NPs (metallic nanoparticles) are the predominant INPs formulated, furthermore, all these nanocarriers are hybrid systems mainly with polymeric nanoparticles, also have properties such as size around 60-278 nm, toxicity less than 40 %, and gene knockdown in a range of 47-90 % *in vitro*. On the other hand, they are mainly used for breast cancer, administered by IV injection. Finally, nanocarriers designed for oral administration of siRNA are presented, proposed by Hosseini, et al. (2020) which were capsules composed of freeze-dried calcium phosphate- polyethylene glycol nanoparticles (CaP-PEG) and trehalose nanoparticles with an outer layer of Eudragit® L100 (EL), chitosan (CS), cellulose acetate phthalate (CAP), hydroxypropyl methylcellulose (HPMC) or/and polyvinyl alcohol (PVA) as an enteric coating. These nanocarriers had a size range of 45 and 65 nm, PDI of 0.16-0.40, potential ζ of 16-18 mV, EGFP knockdown of 21-43 % *in vitro* in HeLa cells, and significant toxicity of around 50-20 % attributed to some polymers used as mucoadhesive excipients.

Table 4: Nanocarriers composed of inorganic nanoparticles

Nanocarrier	Route	Size (nm)	ζ (mV)	Nanocarrier toxicity	Gene silencing	Reference
Magnetic nanoparticles-base / breast cancer	PR	197 ± 16	− 7.8 ± 0.39	MCF-7 cells: < 20 %	No data	Amani et al., 2021
Components: PLA-CS-spermine, PLA-PEG-FA, PLA-PEG-17 peptide, OA-Fe_3O_4, and PTX						
Ceramic nanoparticles-base / ovarian cancer	PR	~91	No data	A2780/AD: <5 %	Bcl-2: 80% *in vitro*	Chen et al., 2009
Components: MSN, PANAM G2, and DOX						
Ceramic nanoparticles-base / endocervical adenocarcinoma	PR	278	-7.3	KB-V1 cells: ~40 %	P-gp: 80-90% *in vitro*	Meng et al., 2010
Components: fosfonato-MSN, PEI, and DOX						
Ceramic nanoparticles-base / oral cancer	ITI	170 ± 3.8	34.7 ± 0.9	KBV cells: ~20 %	MDR1: ~70% *in vitro*	Wang et al., 2017
Components: MSN, PEI, and DOX						
Semiconductor nanoparticles-base / breast cancer	IV	171.7 ± 4	-2.7 ± 0.6	MDA-MB-231 cells: < 20 %	Bcl-2: > 80% *in vivo*	Kim et al., 2019b
Components: Cationic lipid, CdSe/ZnS (QDs), DSPE-mPEG and aptamer						
Semiconductor nanoparticles-base / hepatic cancer	PR	83 ± 3	> 30	SK-Hep1 cells: < 20 %	IL-8: ~ 63% *in vitro*	Cao et al., 2019
Components: PCL- PDEM and CdSe/ZnS (QDs)						
Metallic nanoparticles-base / breast cancer	IT	60 - 120	20 -30	MDA-MB-231 cells: 20 – 40 %	EGFP-Luc: 50 -80% *in vitro*	Taschauer et al., 2020
	IT	87	~ 25			
Components: Au-NP y LPEI (1) / Au-NP y LPEI-PEG						
Metallic nanoparticles-base / melanoma	PR	187 - 228	15-20	B16-F10 cells: < 20 %	PD-L1: 47% *in vitro* PD-L1: 59% *in vivo*	Xue et al., 2021b
Components: Au-NP, PANAM-G5, mPEG-SCM, and fluorescamine						
Metallic nanoparticles-base / lung cancer	PR	86 ± 4	33 ± 3	H1299 cells: < 5 %	GFP: ~70% *in vitro*	Shaabani et al., 2021
Components: AuNP and chitosan						
Metallic nanoparticles-base / breast cancer	IV	128	~ -16	MCF-7 cells: < 5 %	PLK1: 83% *in vitro*	Xue et al., 2021a
Components: Au-NP, ADC and Aptamer-YTDB						
Metallic nanoparticles-base / melanoma	IV	< 100	~ 2	B16-F10 cells: < 10 %	Bcl-2: > 75% *in vitro*	Qiao et al., 2021
Components: CTND-NP (copper complex) and PEI-PEG-FPBA						

IV: Intravenous injection, IT: intratracheal, PR: parenteral route, ITI: Intratumoral injection

SUPPLEMENTARY PERSPECTIVE FOR NANOCARRIER DEVELOPMENT

The success of siRNA-based therapeutics depends largely on their delivery system, thus requiring the use of nanocarriers that are at least: (i) biocompatible, biodegradable and non-immunogenic/non-toxic, (ii) non-sensitive to serum nucleases during transit through systemic circulation, (iii) specific for target cells while avoiding other tissues, and (iv) able to enter the cell membrane, the cellular environment and the endosomal pathway (Ge et al., 2021; Tenchov et al., 2021; Sharma et al., 2020; Mahmoodi Chalbatani et al., 2019).

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The foregoing nanocarriers showed propitious characteristics conducive to siRNA delivery, in general, it was observed that almost all of them are designed for IV administration, regardless of the target. Although IV administration allows 100 % bioavailability of the API, it also had several limitations related to the invasiveness of the API administration process (pain at the injection site, patient discomfort, allergic reactions, scarring, etc.), this aspect should be considered especially for the treatment of chronic degenerative diseases such as cancer, which is one of the main approaches for the use of siRNA as a treatment and/or adjuvant, hence it is necessary that the development of nanocarriers also focuses on an oral administration of siRNA. In addition, this kind of administration can represent a potent modality for treating many gastrointestinal diseases such as inflammatory bowel disease (IBD), irritable bowel syndrome, and colon cancer, without adverse systemic effects (Tran and Park, 2021; El-Mayta et al., 2021; Kanugo and Misra, 2020).

It is well known that oral administration requires several efforts to deliver APIs, even more than parenteral administration (see Figure 3), since it is a complex process that can be affected by different factors such as physiological and cellular barriers, in particular, it was shown in some studies that naked siRNA can withstand gastric challenges for one hour at physiological temperature, but is inevitably degraded by nucleases, thus siRNA necessarily needs a nanocarrier that can avoid enzymatic digestion, overcome GI mucus barriers, and facilitate their delivery into target cells (Rehman et al., 2021; Ruiz-Picazo et al., 2021).

Some nanocarriers have been studied for oral siRNA administration; these nanocarriers are mainly composed of polymers and lipids. An example of oral administration was proposed by (Wang et al., 2021), they formed a lipoplex with folic acid-conjugated ginger-derived lipid and siRNA. Although polymers are good absorption enhancers (~bioavailability) and have benefits such as controlled drug release, they cannot provide a satisfactory solution due to their associated toxicities, so lipid-based drug delivery systems (LBDDS) have been frequently proposed in recent years. These nanocarriers have advantages such as low toxicity, low cost, affordable scale-up manufacture, high biocompatibility, high drug loading efficiency and recruit a range of lipid digestion pathways in the GI tract that play a decisive role in the drug absorption process (Ashkar et al., 2022; Plaza-Oliver et al., 2021; Zu et al., 2021; Tran et al., 2018).

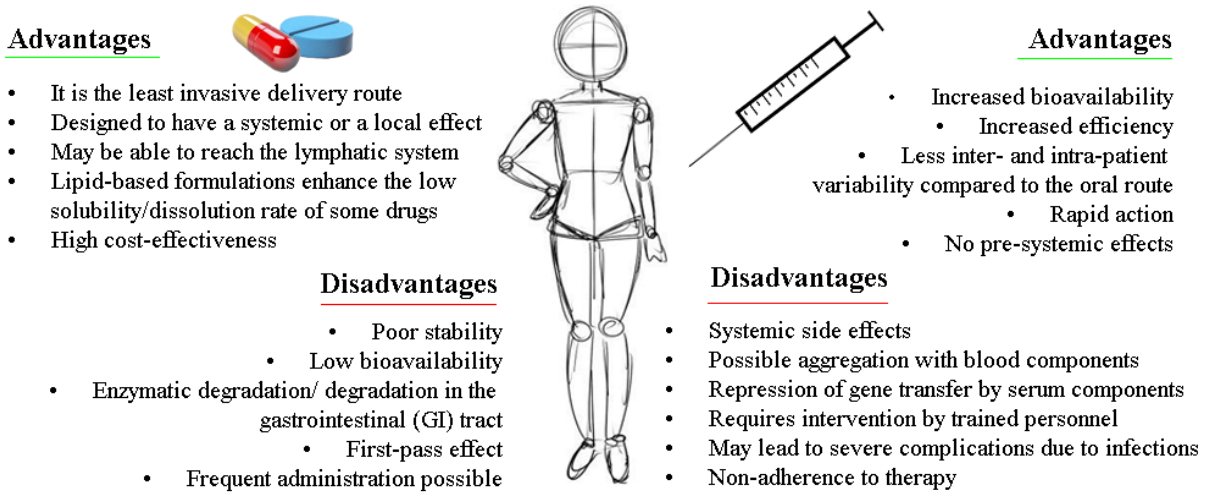

Figure 3: Advantage and disadvantage in oral and intravenous route for siRNA delivery, (Antimisiaris et al., 2021; Fumoto et al., 2021; van den Berg et al., 2021; Lorscheider et al., 2021; Hanna and Mayden, 2021)

LBDDS can be classified into three types, previously mentioned two types: vesicular systems (micelles and liposomes) and solid lipid systems, the last type is an emulsion system, which is a novel approach for oral siRNA administration, especially the self-nanoemulsifying drug delivery system (SNEDDS). This system is composed of dissolved API, long and/or medium-chain triglyceride oils, high concentrations of non-ionic surfactants with HLB>12, and co-solvents to reduce interface between the oil and the aqueous medium, spontaneously forming fine oil-in-water nanoemulsions (o/w) *in situ* in the GI tract thanks to the stomach and small intestine motility (peri-stalsis) and the aqueous medium of the GI fluids, in a process called self-nanoemulsion (Dalal et al., 2021; Xu, et al., 2021b; Morakul, 2020; Sokkula and Gande, 2020; Knaub et al., 2019; Krstić et al., 2018; Cherniakov et al., 2015).

SNEDDS is an ideally isotropic and thermodynamically stable mixture, with droplet sizes below 200 nm thus having a large interfacial surface area for dispersion into the GI fluid, it is mainly designed to increase the solubility and permeability of APIs with lipophilic characteristics, however it has recently started to be used to improve the oral administration of hydrophilic macromolecules such as siRNA (nucleic acids), in such a way that the rate of drug dissolution, its absorption, digestion, and bioavailability can be improved. In addition, SNEDDS has a high drug loading capacity, is easy to manufacture and scale-up, it has good kinetic stability after dispersion in an aqueous medium, requiring a minimum amount of energy for dispersion and preparation, it has high physical stability during storage, decreases the first-pass effect and enhances penetration of highly lipophilic APIs into the intestinal membrane through the recruitment of intestinal lymphatic transport (Okonogi et al., 2021; Jain et al., 2021; Mehanna and Mneimneh 2020; Buya et al., 2020; Cardona et al., 2019; Gilani et al., 2019; Ng and Rogers, 2019; Rehman et al., 2017).

The main strategy for incorporating nucleic acids into SNEDDS includes reducing their hydrophilicity by pairing hydrophobic ions, this method is based on replacing the negatively charged counterions with positively charged surfactants or cationic lipids. The first work on this was presented by (Hauptstein et al., 2015), where pDNA complexes were formed using 5 different cationic components highlighting the use of cetrimide. These complexes were properly dissolved in SNEDDS thus the pDNA was successfully incorporated, obtaining a nanocarrier with an effect against enzymatic degradation and a good transfection efficiency of HEK-293 cells. Furthermore, Mahmood et al. (2016) presented a similar work based on pDNA-cetrimide, where the transfection efficiency of SNEDDS was improved by the incorporation of a cell-penetrating peptide (TAT-OL). Finally, the most recent work, to our knowledge, was presented by Kubackova et al. (2021) where SEDDS loaded with oligonucleotide (OND)-DDAB or DOTAP complexes were prepared and characterized using the hydrophobic ion pairing technique. This nanocarrier was a viable delivery system across the Caco-2 monolayer and was protected OND in the GI tract.

CONCLUSIONS

The use of siRNA as a mediator of gene silencing is a novel alternative for the treatment of various diseases, its advantages over traditional RNA delivery make it a suitable tool for the improvement of the bioavailability of a therapeutic effect. As a result, a wide range of nanocarriers for the transport and delivery of siRNA has been developed, however, only a few of them are in clinical trials.

The classification of nanocarriers outlined in this review is a suggestion, which considers the nature (organic and inorganic) of single ingredients, their chemical structure (lipids and polymers), and the shape of the nanocarrier (liposomes/polymersomes and micelles). However, almost all nanocarriers are hybrid

systems and should not be limited to a single classification; a relevant example of this are inorganic nanocarriers, which are generally composed of siRNA complexes with organic nanoparticles.

These nanocarriers have proven to be stable, biocompatible, and effective *in vitro*, but only very few are designed for oral administration of siRNA. This approach has emerged to offer enhanced nanocarriers that can satisfy different needs, such as a targeted treatment for gastrointestinal diseases and nanocarriers that may facilitate adherence to treatments and do not affect the patients' quality of life. Therefore, there is a need to further explore the development of nanocarriers to obtain safe, biocompatible, and suitable biopharmaceutical tools that allow the enhancement of the absorption and targeting of siRNA for effective therapeutic alternatives.

Declaration of competing interests

The authors declare no conflict of interest.

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REFERENCES

Aghamiri S, Raee P, Talaei S, Mohammadi-Yeganeh S, Bayat S, Rezaee D, et al. Nonviral siRNA delivery systems for pancreatic cancer therapy. Biotechnol Bioeng. 2021;118:3669–90.

Ahmed A, Sarwar S, Hu Y, Munir MU, Nisar MF, Ikram F, et al. Surface-modified polymeric nanoparticles for drug delivery to cancer cells. Expert Opin Drug Deliv. 2021a;18(1):1–24.

Ahmed S, Salmon H, Distasio N, Doan H, Scherman D, Alhareth K, et al. Viscous core liposomes increase sirna encapsulation and provides gene inhibition when slightly positively charged. Pharmaceutics. 2021b;13 (4):479.

Ajeeshkumar KK, Aneesh PA, Raju N, Suseela M, Ravishankar CN, Benjakul S. Advancements in liposome technology: Preparation techniques and applications in food, functional foods, and bioactive delivery: A review. Compr Rev Food Sci Food Saf. 2021;20: 1280–306.

Aldosari BN, Alfagih IM, Almurshedi AS. Lipid nanoparticles as delivery systems for RNA-based vaccines. Pharmaceutics. 2021;13(2):206.

Amani A, Dustparast M, Noruzpour M, Zakaria RA, Ebrahimi HA. Design and invitro characterization of green synthesized magnetic nanoparticles conjugated with multitargeted poly lactic acid copolymers for co-delivery of siRNA and paclitaxel. Eur J Pharm Sci. 2021;167:106007.

Antimisiaris SG, Marazioti A, Kannavou M, Natsaridis E, Gkartziou F, Kogkos G, et al. Overcoming barriers by local drug delivery with liposomes. Adv Drug Deliv Rev. 2021;174:53–86.

Araste F, Aliabadi A, Abnous K, Taghdisi SM, Ramezani M, Alibolandi M. Self-assembled polymeric vesicles: Focus on polymersomes in cancer treatment. J Control Release. 2021;330:502–28.

Ashkar A, Sosnik A, Davidovich-Pinhas M. Structured edible lipid-based particle systems for oral drug-delivery. Biotechnol Adv. 2022;54:107789.

Ashrafizadeh M, Hushmandi K, Rahmani Moghadam E, Zarrin V, Hosseinzadeh Kashani S, Bokaie S, et al. Progress in delivery of siRNA-based therapeutics employing nano-vehicles for treatment of prostate cancer. Bioengineering. 2020;7(3):91.

Bai G, Xue T, Dong X, Chinta UK, Feng J, Jin T, et al. Assembling structurally customizable synthetic carriers of siRNA through thermodynamically self-regulated process. Asian J Pharm Sci. 2020;15:356–64.

Ball RL, Bajaj P, Whitehead KA. Oral delivery of siRNA lipid nanoparticles: Fate in the GI tract. Sci Rep. 2018;8(1):2178.

Basha SK, Dhandayuthabani R, Muzammil MS, Kumari VS. Solid lipid nanoparticles for oral drug delivery. Mater Today Proc. 2021;36:313–24.

Bassetto M, Sen M, Poulhes F, Arango-Gonzalez B, Bonvin E, Sapet C, et al. New method for efficient sirna delivery in retina explants: reverse magnetofection. Bioconjug Chem. 2021;32:1078–93.

Berger M, Lechanteur A, Evrard B, Piel G. Innovative lipoplexes formulations with enhanced siRNA efficacy for cancer treatment: Where are we now? Int J Pharm. 2021;605:120851.

Bholakant R, Qian H, Zhang J, Huang X, Huang D, Feijen J, et al. Recent advances of polycationic siRNA vectors for cancer therapy. Biomacromolecules. 2020; 21:2966–82.

Buya AB, Beloqui A, Memvanga PB, Préat V. Self-nano-emulsifying drug-delivery systems: from the development to the current applications and challenges in oral drug delivery. Pharmaceutics. 2020;12(12): 1194.

Cao N, Cheng D, Zou S, Ai H, Gao J, Shuai X. The synergistic effect of hierarchical assemblies of siRNA and chemotherapeutic drugs co-delivered into hepatic cancer cells. Biomaterials. 2011;32:2222–32.

Cao Z, Xiao H, Li L, Liu M, Lin G, Zhai P, et al. The codelivery of siRNA and QDs by pH-responsive micelle for hepatoma cancer cells. Front Pharmacol. 2019;10:1194.

Cardona MI, Nguyen Le N-M, Zaichik S, Aragón DM, Bernkop-Schnürch A. Development and in vitro characterization of an oral self-emulsifying delivery system (SEDDS) for rutin fatty ester with high mucus permeating properties. Int J Pharm. 2019;562:180–6.

Castro E, Kumar A. Nanoparticles in drug delivery systems. In: Kumar A, Mansour HM, Friedman A, Blough ER (eds): Nanomedicine in drug delivery (pp 1-22). Boca Raton, FL: CRC Press, 2013.

Castro KC de, Costa JM, Campos MGN. Drug-loaded polymeric nanoparticles: a review. Int J Polym Mater Polym Biomater. 2022;71(1):1–13.

Ceborska M. Folate appended cyclodextrins for drug, DNA, and siRNA delivery. Eur J Pharm Biopharm. 2017;120:133–45.

Charbe NB, Amnerkar ND, Ramesh B, Tambuwala MM, Bakshi HA, Aljabali AAA, et al. Small interfering RNA for cancer treatment: overcoming hurdles in delivery. Acta Pharm Sin B. 2020;10:2075-109.

Chen AM, Zhang M, Wei D, Stueber D, Taratula O, Minko T, et al. Co-delivery of doxorubicin and Bcl-2 siRNA by mesoporous silica nanoparticles enhances the efficacy of chemotherapy in multidrug-resistant cancer cells. Small. 2009;5(23):2673–7.

Chen J, Liu Q, Xiao J, Du J. EpCAM-antibody-labeled noncytotoxic polymer vesicles for cancer stem cells-targeted delivery of anticancer drug and siRNA. Biomacromolecules. 2015;16:1695–705.

Chen W, Yuan Y, Cheng D, Chen J, Wang L, Shuai X. Co-Delivery of doxorubicin and siRNA with reduction and ph dually sensitive nanocarrier for synergistic cancer therapy. Small. 2014;10:2678–87.

Chenthamara D, Subramaniam S, Ramakrishnan SG, Krishnaswamy S, Essa MM, Lin F-H, et al. Therapeutic efficacy of nanoparticles and routes of administration. Biomater Res. 2019;23(1):20.

Cherniakov I, Domb AJ, Hoffman A. Self-nano-emulsifying drug delivery systems: an update of the biopharmaceutical aspects. Expert Opin Drug Deliv 2015; 12:1121–33.

Chevalier R. siRNA targeting and treatment of gastrointestinal diseases. Clin Transl Sci. 2019;12: 573–85.

Craparo EF, Drago SE, Mauro N, Giammona G, Cavallaro G. Design of new polyaspartamide copolymers for siRNA delivery in antiasthmatic therapy. Pharmaceutics. 2020;12(2):89.

Cullis PR, Hope MJ. Lipid nanoparticle systems for enabling gene therapies. Mol Ther. 2017;25:1467–75.

Dalal L, Allaf AW, El-Zein H. Formulation and in vitro evaluation of self-nanoemulsifying liquisolid tablets of furosemide. Sci Rep. 2021;11(1):1315.

Dhiman N, Awasthi R, Sharma B, Kharkwal H, Kulkarni GT. Lipid nanoparticles as carriers for bioactive delivery. Front Chem. 2021;9:580118.

Dowaidar M, Abdelhamid HN, Hällbrink M, Freimann K, Kurrikoff K, Zou X, et al. Magnetic nanoparticle assisted self-assembly of cell penetrating peptides-oligonucleotides complexes for gene delivery. Sci Rep. 2017;7(1):9159.

El-Mayta R, Zhang R, Shepherd SJ, Wang F, Billingsley MM, Dudkin V, et al. A Nanoparticle platform for accelerated in vivo oral delivery screening of nucleic acids. Adv Ther. 2021;4(1):2000111.

Fairman K, Li M, Ning B, Lumen A. Physiologically based pharmacokinetic (PBPK) modeling of RNAi therapeutics: Opportunities and challenges. Biochem Pharmacol. 2021;189:114468.

Ferenchak K, Deitch I, Huckfeldt R. Antisense oligonucleotide therapy for ophthalmic conditions. Semin Ophthalmol. 2021;36:452–7.

Fliervoet LAL, Zhang H, van Groesen E, Fortuin K, Duin NJCB, Remaut K, et al. Local release of siRNA using polyplex-loaded thermosensitive hydrogels. Nanoscale. 2020;12:10347–60.

Fumoto S, Yamamoto T, Okami K, Maemura Y, Terada C, Yamayoshi A, et al. Understanding in vivo fate of nucleic acid and gene medicines for the rational design of drugs. Pharmaceutics. 2021;13(2):159.

Gallon E, Matini T, Sasso L, Mantovani G, Armiñan de Benito A, Sanchis J, et al. Triblock copolymer nanovesicles for pH-responsive targeted delivery and controlled release of siRNA to cancer cells. Biomacromolecules. 2015;16:1924–37.

Gao H, Cheng R, Santos HA. Nanoparticle-mediated siRNA delivery systems for cancer therapy. VIEW. 2021a;2(3):20200111.

Gao L, Yu S, Lin J, Chen S, Shen Z, Kang M. Construction of PD-L1-siRNA and IL-2 DNA loading PEI lipid nanoparticles in activating T cells and treating lung cancer. AIP Adv. 2021b;11(4):045201.

García-Fernández A, Sancenón F, Martínez-Máñez R. Mesoporous silica nanoparticles for pulmonary drug delivery. Adv Drug Deliv Rev. 2021;177:113953.

Ge X, Chen L, Zhao B, Yuan W. Rationale and application of PEGylated lipid-based system for advanced target delivery of siRNA. Front Pharmacol. 2021;11:598175.

Ghezzi M, Pescina S, Padula C, Santi P, Del Favero E, Cantù L, et al. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. J Control Release. 2021;332:312–36.

Gidwani B, Sahu V, Shukla SS, Pandey R, Joshi V, Jain VK, et al. Quantum dots: Prospectives, toxicity, advances and applications. J Drug Deliv Sci Technol. 2021;61:102308.

Gilani SJ, Rizwanullah M, Imam SS, Pandit J, Aqil M, Alam M, et al. QbD considerations for topical and transdermal product development. In: Beg S, Hasnain MS: Pharmaceutical quality by design: principles and applications (pp 131-50). Amsterdam: Elsevier, 2019.

Gumala A, Sutriyo S. Active targeting gold nanoparticle for chemotherapy drug delivery: A Review. Pharm Sci. 2021; epub ahead of print. doi: 10.34172/PS.2021.75.

Habib S, Daniels A, Ariatti M, Singh M. Anti-c-myc cholesterol based lipoplexes as onco-nanotherapeutic agents in vitro. F1000Res. 2021;9:770.

Han X, Zhang H, Butowska K, Swingle KL, Alameh M-G, Weissman D, et al. An ionizable lipid toolbox for RNA delivery. Nat Commun. 2021;12(1):7233.

Hanna K, Mayden K. The use of real-world evidence for oral chemotherapies in breast cancer. J Adv Pract Oncol. 2021;12(Suppl 2):13-20.

Hattori Y, Tamaki K, Sakasai S, Ozaki K, Onishi H. Effects of PEG anchors in PEGylated siRNA lipoplexes on in vitro gene-silencing effects and siRNA biodistribution in mice. Mol Med Rep. 2020;22:4183-96.

Hauptstein S, Prüfert F, Bernkop-Schnürch A. Self-nanoemulsifying drug delivery systems as novel approach for pDNA drug delivery. Int J Pharm. 2015; 487:25–31.

He C, Liu D, Lin W. Self-assembled nanoscale coordination polymers carrying siRNAs and cisplatin for effective treatment of resistant ovarian cancer. Biomaterials. 2015;36:124–33.

He C, Yue H, Xu L, Liu Y, Song Y, Tang C, et al. siRNA release kinetics from polymeric nanoparticles correlate with RNAi efficiency and inflammation therapy via oral delivery. Acta Biomater. 2020;103: 213–22.

Hodgson J. Refreshing the biologic pipeline 2020. Nat Biotechnol. 2021;39:135–43.

Hosseini S, Wey K, Epple M. Enteric coating systems for the oral administration of bioactive calcium phosphate nanoparticles carrying nucleic acids into the colon. ChemistrySelect. 2020;5(31):9720–9.

Huang R-Y, Liu Z-H, Weng W-H, Chang C-W. Magnetic nanocomplexes for gene delivery applications. J Mater Chem B. 2021;9:4267–86.

Ibaraki H, Kanazawa T, Owada M, Iwaya K, Takashima Y, Seta Y. Anti-metastatic effects on melanoma via intravenous administration of anti-NF-κB siRNA complexed with functional peptide-modified nano-micelles. Pharmaceutics. 2020;12(1): 64.

Inglut CT, Sorrin AJ, Kuruppu T, Vig S, Cicalo J, Ahmad H, et al. Immunological and toxicological considerations for the design of liposomes. Nanomaterials. 2020;10(2):190.

Iqbal S, Blenner M, Alexander-Bryant A, Larsen J. Polymersomes for therapeutic delivery of protein and nucleic acid macromolecules: from design to therapeutic applications. Biomacromolecules. 2020;21:1327–50.

Itani R, Al Faraj A. siRNA conjugated nanoparticles - a next generation strategy to treat lung cancer. Int J Mol Sci. 2019;20(23):6088.

Jain V, Kumar H, Chand P, Jain S, S P. Lipid-based nanocarriers as drug delivery system and its applications. In: Dave V, Gupta N, Sur S (eds): Nanopharmaceutical advanced delivery systems (pp 1-29). Beverly, MA: Scrivener Publishing LLC, 2021.

Jiang Z, Thayumanavan S. Noncationic material design for nucleic acid delivery. Adv Ther. 2020;3(3): 1900206.

Jyotsana N, Sharma A, Chaturvedi A, Budida R, Scherr M, Kuchenbauer F, et al. Lipid nanoparticle-mediated siRNA delivery for safe targeting of human CML in vivo. Ann Hematol. 2019;98:1905–18.

Kala S, Mak ASC, Liu X, Posocco P, Pricl S, Peng L, et al. Combination of dendrimer-nanovector-mediated small interfering RNA delivery to target akt with the clinical anticancer drug paclitaxel for effective and potent anticancer activity in treating ovarian cancer. J Med Chem. 2014;57:2634–42.

Kaneshiro TL, Lu Z-R. Targeted intracellular codelivery of chemotherapeutics and nucleic acid with a well-defined dendrimer-based nanoglobular carrier. Biomaterials. 2009;30:5660–6.

Kanugo A, Misra A. New and novel approaches for enhancing the oral absorption and bioavailability of protein and peptides therapeutics. Ther Deliv. 2020;11: 713–32.

Khalid K, Tan X, Mohd Zaid HF, Tao Y, Lye Chew C, Chu D-T, et al. Advanced in developmental organic and inorganic nanomaterial: a review. Bioengineered. 2020;11:328–55.

Khan MA, Singh D, Ahmad A, Siddique HR. Revisiting inorganic nanoparticles as promising therapeutic agents: A paradigm shift in oncological theranostics. Eur J Pharm Sci. 2021;164:105892.

Khurana A, Tekula S, Saifi MA, Venkatesh P, Godugu C. Therapeutic applications of selenium nanoparticles. Biomed Pharmacother. 2019;111:802–12.

Kim B, Park J, Sailor MJ. Rekindling RNAi therapy: materials design requirements for in vivo siRNA delivery. Adv Mater. 2019a;31(49):1903637.

Kim H-O, Kim E, An Y, Choi J, Jang E, Choi EB, et al. A biodegradable polymersome containing bcl-xl sirna and doxorubicin as a dual delivery vehicle for a synergistic anticancer effect. Macromol Biosci. 2013; 13:745–54.

Kim MW, Jeong HY, Kang SJ, Choi MJ, You YM, Im CS, et al. Cancer-targeted nucleic acid delivery and quantum dot imaging using EGF receptor aptamer-conjugated lipid nanoparticles. Sci Rep. 2017;7(1): 9474.

Kim MW, Jeong HY, Kang SJ, Jeong IH, Choi MJ, You YM, et al. Anti-EGF receptor aptamer-guided co-delivery of anti-cancer siRNAs and quantum dots for theranostics of triple-negative breast cancer. Theranostics. 2019b;9:837–52.

Kim Y, Tewari M, Pajerowski JD, Cai S, Sen S, Williams J, et al. Polymersome delivery of siRNA and antisense oligonucleotides. J Control Release. 2009; 134:132–40.

Kim YH, Lee K, Li S. Nucleic acids based polyelectrolyte complexes: their complexation mechanism, morphology, and stability. Chem Mater. 2021;33: 7923–43.

Knaub K, Sartorius T, Dharsono T, Wacker R, Wilhelm M, Schön C. A novel self-emulsifying drug delivery system (SEDDS) based on VESIsorb® formulation technology improving the oral bioavailability of cannabidiol in healthy subjects. Molecules. 2019;24 (16):2967.

Kokkinos J, Ignacio RMC, Sharbeen G, Boyer C, Gonzales-Aloy E, Goldstein D, et al. Targeting the undruggable in pancreatic cancer using nano-based gene silencing drugs. Biomaterials. 2020;240:119742.

Koynova R, Caffrey M. Phases and phase transitions of the phosphatidylcholines. Biochim Biophys Acta. 1998;1376:91–145.

Krstić M, Medarević Đ, Đuriš J, Ibrić S. Self-nanoemulsifying drug delivery systems (SNEDDS) and self-microemulsifying drug delivery systems (SMEDDS) as lipid nanocarriers for improving dissolution rate and bioavailability of poorly soluble drugs. In: Grumezescu AM (ed): Lipid nanocarriers for drug targeting (pp 473-508). Amsterdam: Elsevier, 2018.

Kubackova J, Holas O, Zbytovska J, Vranikova B, Zeng G, Pavek P, et al. Oligonucleotide delivery across the Caco-2 monolayer: the design and evaluation of self-emulsifying drug delivery systems (SEDDS). Pharmaceutics. 2021;13(4):459.

Lee D-J, Kessel E, Edinger D, He D, Klein PM, Voith von Voithenberg L, et al. Dual antitumoral potency of EG5 siRNA nanoplexes armed with cytotoxic bifunctional glutamyl-methotrexate targeting ligand. Biomaterials. 2016;77:98–110.

Li F, Yu W, Zhang J, Dong Y, Ding X, Ruan X, et al. Spatiotemporally programmable cascade hybridization of hairpin DNA in polymeric nanoframework for precise siRNA delivery. Nat Commun. 2021a;12(1): 1138.

Li X, Li W, Wang M, Liao Z. Magnetic nanoparticles for cancer theranostics: Advances and prospects. J Control Release. 2021b;335:437–48.

Li Z, Li J, Huang J, Zhang J, Cheng D, Shuai X. Synthesis and characterization of pH-responsive copolypeptides vesicles for siRNA and chemotherapeutic drug co-delivery. Macromol Biosci. 2015;15: 1497–50.

Lin Y-X, Wang Y, Blake S, Yu M, Mei L, Wang H, et al. RNA nanotechnology-mediated cancer immunotherapy. Theranostics. 2020;10:281–99.

Lins P, Ribovski L, Sampaio I, Santos O, Zucolotto V, Cancino-Bernardi J. Inorganic nanoparticles for biomedical applications. In: Eloy JO, Abriata JP, Marchetti JM (eds): Nanocarriers for drug delivery: concepts and applications (pp 49-72). Cham: Springer International Publ., 2021.

Liu Y, Cao F, Sun B, Bellanti JA, Zheng SG. Magnetic nanoparticles: A new diagnostic and treatment platform for rheumatoid arthritis. J Leukoc Biol. 2021a; 109:415–24.

Liu Z, Ye L, Xi J, Wang J, Feng Z. Cyclodextrin polymers: Structure, synthesis, and use as drug carriers. Prog Polym Sci. 2021b;118:101408.

Lorscheider M, Gaudin A, Nakhlé J, Veiman K-L, Richard J, Chassaing C. Challenges and opportunities in the delivery of cancer therapeutics: update on recent progress. Ther Deliv. 2021;12(1):55–76.

Lu Y, Zhong L, Jiang Z, Pan H, Zhang Y, Zhu G, et al. Cationic micelle-based siRNA delivery for efficient colon cancer gene therapy. Nanoscale Res Lett. 2019; 14(1):193.

Mahmood A, Prüfert F, Efiana NA, Ashraf MI, Hermann M, Hussain S, et al. Cell-penetrating self-nanoemulsifying drug delivery systems (SNEDDS) for oral gene delivery. Expert Opin Drug Deliv. 2016;13: 1503–12.

Mahmoodi Chalbatani G, Dana H, Gharagouzloo E, Grijalvo S, Eritja R, Logsdon CD, et al. Small interfering RNAs (siRNAs) in cancer therapy: a nano-based approach. Int J Nanomedicine. 2019;14:3111–28.

Mainini F, Eccles MR. Lipid and polymer-based nanoparticle siRNA delivery systems for cancer therapy. molecules. 2020;25(11):2692.

Majumder J, Minko T. Multifunctional and stimuli-responsive nanocarriers for targeted therapeutic delivery. Expert Opin Drug Deliv. 2021;18:205–27.

Maurer V, Altin S, Ag Seleci D, Zarinwall A, Temel B, Vogt PM, et al. In-vitro application of magnetic hybrid niosomes: targeted siRNA-delivery for enhanced breast cancer therapy. Pharmaceutics. 2021; 13(3):394.

Mehanna MM, Mneimneh AT. Formulation and applications of lipid-based nanovehicles: spotlight on self-emulsifying systems. Adv Pharm Bull. 2020;11 (1):56–67.

Meng H, Liong M, Xia T, Li Z, Ji Z, Zink JI, et al. Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and p-glycoprotein siRNA to overcome drug resistance in a cancer cell line. ACS Nano. 2010;4:4539–50.

Misra R, Das M, Sahoo BS, Sahoo SK. Reversal of multidrug resistance in vitro by co-delivery of MDR1 targeting siRNA and doxorubicin using a novel cationic poly(lactide-co-glycolide) nanoformulation. Int J Pharm. 2014;475:372–84.

Moore JA, Chow JCL. Recent progress and applications of gold nanotechnology in medical biophysics using artificial intelligence and mathematical modeling. Nano Express. 2021;2(2):022001.

Morakul B. Self-nanoemulsifying drug delivery systems (SNEDDS): an advancement technology for oral drug delivery. Pharm Sci Asia. 2020;47:205–20.

Moulahoum H, Ghorbanizamani F, Zihnioglu F, Timur S. Surface biomodification of liposomes and polymersomes for efficient targeted drug delivery. Bioconjug Chem. 2021;32:1491–502.

Mousazadeh H, Pilehvar-Soltanahmadi Y, Dadashpour M, Zarghami N. Cyclodextrin based natural nanostructured carbohydrate polymers as effective non-viral siRNA delivery systems for cancer gene therapy. J Control Release. 2021;330:1046–70.

Ng N, Rogers MA. Surfactants. In: Melton L, Shahidi F, Varelis P (eds): Encyclopedia of food chemistry, Vol. 1 (pp 276-82). Amsterdam: Elsevier, 2019.

Noh SM, Han SE, Shim G, Lee KE, Kim C-W, Han SS, et al. Tocopheryl oligochitosan-based self assembling oligomersomes for siRNA delivery. Biomaterials. 2011;32:849–57.

Noske S, Karimov M, Aigner A, Ewe A. Tyrosine-modification of polypropylenimine (PPI) and polyethylenimine (PEI) strongly improves efficacy of siRNA-mediated gene knockdown. Nanomaterials. 2020;10 (9):1809.

Ojo OA, Olayide II, Akalabu MC, Ajiboye BO, Ojo AB, Oyinloye BE, et al. Nanoparticles and their biomedical applications. Biointerface Res Appl Chem. 2021;11:8431–45.

Okonogi S, Phumat P, Khongkhunthian S, Chaijareenont P, Rades T, Müllertz A. Development of self-nanoemulsifying drug delivery systems containing 4-allylpyrocatechol for treatment of oral infections caused by Candida albicans. Pharmaceutics. 2021;13 (2):167.

Pan J, Mendes LP, Yao M, Filipczak N, Garai S, Thakur GA, et al. Polyamidoamine dendrimers-based nanomedicine for combination therapy with siRNA and chemotherapeutics to overcome multidrug resistance. Eur J Pharm Biopharm. 2019;136:18–28.

Pandey A. Cyclodextrin-based nanoparticles for pharmaceutical applications: a review. Environ Chem Lett. 2021;19:4297–310.

Pandey M, Rani P, Adhikari L, Gupta M, Semalty A, Semalty M. Preparation and characterization of cyclodextrin complexes of doxycycline hyclate for improved photostability in aqueous solution. J Incl Phenom Macrocycl Chem. 2022;102:271–8.

Pangburn TO, Georgiou K, Bates FS, Kokkoli E. Targeted polymersome delivery of siRNA induces cell death of breast cancer cells dependent upon Orai3 protein expression. Langmuir. 2012;28:12816–30.

Patel P, Ibrahim NM, Cheng K. The importance of apparent pKa in the development of nanoparticles encapsulating siRNA and mRNA. Trends Pharmacol Sci. 2021;42:448–60.

Paunovska K, Loughrey D, Dahlman JE. Drug delivery systems for RNA therapeutics. Nat Rev Genet. 2022; 23:265-80.

Petitjean M, García-Zubiri IX, Isasi JR. History of cyclodextrin-based polymers in food and pharmacy: a review. Environ Chem Lett. 2021;19:3465–76.

Pishavar E, Oroojalian F, Salmasi Z, Hashemi E, Hashemi M. Recent advances of dendrimer in targeted delivery of drugs and genes to stem cells as cellular vehicles. Biotechnol Prog. 2021;37(4):e3174.

Plaza-Oliver M, Santander-Ortega MJ, Lozano MV. Current approaches in lipid-based nanocarriers for oral drug delivery. Drug Deliv Transl Res. 2021;11:471–97.

Pylaev T, Avdeeva E, Khlebtsov N. Plasmonic nanoparticles and nucleic acids hybrids for targeted gene delivery, bioimaging, and molecular recognition. J Innov Opt Health Sci. 2021;14(04):2130003.

Qiao H, Zhang L, Fang D, Zhu Z, He W, Hu L, et al. Surmounting tumor resistance to metallodrugs by co-loading a metal complex and siRNA in nanoparticles. Chem Sci. 2021;12:4547–56.

Qiu M, Ouyang J, Wei Y, Zhang J, Lan Q, Deng C, et al. Selective cell penetrating peptide ‐ functionalized envelope ‐ type chimeric lipopepsomes boost systemic RNAi therapy for lung tumors. Adv Healthc Mater. 2019;8(16):1900500.

Qu M-H, Zeng R-F, Fang S, Dai Q-S, Li H-P, Long J-T. Liposome-based co-delivery of siRNA and docetaxel for the synergistic treatment of lung cancer. Int J Pharm. 2014;474:112–22.

Rehman AU, Akram S, Seralin A, Vandamme T, Anton N. Lipid nanocarriers: Formulation, properties, and applications. In: Nguyen-Tri P, Do T-O, Nguyen TA (eds): Smart nanocontainers. A volume in micro and nano technologies (pp 355-82). Amsterdam: Elsevier, 2020.

Rehman AU, Busignies V, Coelho Silva Ribeiro M, Almeida Lage N, Tchoreloff P, Escriou V, et al. Fate of tableted freeze-dried siRNA lipoplexes in gastrointestinal environment. Pharmaceutics. 2021;13 (11):1807.

Rehman FU, Shah KU, Shah SU, Khan IU, Khan GM, Khan A. From nanoemulsions to self-nanoemulsions, with recent advances in self-nanoemulsifying drug delivery systems (SNEDDS). Expert Opin Drug Deliv. 2017;14:1325–40.

Ruiz-Picazo A, Lozoya-Agullo I, González-Álvarez I, Bermejo M, González-Álvarez M. Effect of excipients on oral absorption process according to the different gastrointestinal segments. Expert Opin Drug Deliv. 2021;18:1005–24.

Sajid MI, Moazzam M, Kato S, Yeseom Cho K, Tiwari RK. Overcoming barriers for siRNA therapeutics: from bench to bedside. Pharmaceuticals. 2020;13(10):294.

Salzano G, Navarro G, Trivedi MS, De Rosa G, Torchilin VP. Multifunctional polymeric micelles co-loaded with anti–survivin siRNA and paclitaxel overcome drug resistance in an animal model of ovarian cancer. Mol Cancer Ther. 2015;14:1075–84.

Sánchez-Arribas N, Martínez-Negro M, Villar EM, Pérez L, Osío Barcina J, Aicart E, et al. Protein expression knockdown in cancer cells induced by a gemini cationic lipid nanovector with histidine-based polar heads. Pharmaceutics. 2020;12(9):791.

Sarkies P, Miska EA. Small RNAs break out: the molecular cell biology of mobile small RNAs. Nat Rev Mol Cell Biol. 2014;15:525–35.

Scheerstra JF, Wauters AC, Tel J, Abdelmohsen LKEA, van Hest JCM. Polymersomes as a potential platform for cancer immunotherapy. Mater Today Adv. 2022;13:100203.

Scheideler M, Vidakovic I, Prassl R. Lipid nanocarriers for microRNA delivery. Chem Phys Lipids. 2020; 226:104837.

Shaabani E, Sharifiaghdam M, De Keersmaecker H, De Rycke R, De Smedt S, Faridi-Majidi R, et al. Layer by layer assembled chitosan-coated gold nanoparticles for enhanced siRNA delivery and silencing. Int J Mol Sci. 2021;22(2):831.

Sharma A, Jha NK, Dahiya K, Singh VK, Chaurasiya K, Jha AN, et al. Nanoparticulate RNA delivery systems in cancer. Cancer Rep (Hoboken). 2020;3(5): e1271.

Shi K, Zhao Y, Miao L, Satterlee A, Haynes M, Luo C, et al. Dual functional lipoMET mediates envelope-type nanoparticles to combinational oncogene silencing and tumor growth inhibition. Mol Ther. 2017;25:1567–79.

Shin JH, Shin DH, Kim JS. Let-7 miRNA and CDK4 siRNA co-encapsulated in Herceptin-conjugated liposome for breast cancer stem cells. Asian J Pharm Sci. 2020;15:472–81.

Singh A, Trivedi P, Jain NK. Advances in siRNA delivery in cancer therapy. Artif Cells Nanomed Biotechnol. 2018;46:274-83.

Singh A, Mallika TN, Gorain B, Yadav AK, Tiwari S, Flora SJS, et al. Quantum dot: Heralding a brighter future in neurodegenerative disorders. J Drug Deliv Sci Technol. 2021;65:102700.

Smith SA, Selby LI, Johnston APR, Such GK. The endosomal escape of nanoparticles: toward more efficient cellular delivery. Bioconjug Chem. 2019;30: 263–72.

Sokkula SR, Gande S. A comprehensive review on self-nano emulsifying drug delivery systems: advancements & applications. Int J Pharm Sci Drug Res. 2020; 12:576–83.

Subhan MA, Attia SA, Torchilin VP. Advances in siRNA delivery strategies for the treatment of MDR cancer. Life Sci. 2021;274:119337.

Sun T-M, Du J-Z, Yao Y-D, Mao C-Q, Dou S, Huang S-Y, et al. Simultaneous delivery of siRNA and paclitaxel via a "two-in-one" micelleplex promotes synergistic tumor suppression. ACS Nano. 2011;5: 1483–94.

Taleghani AS, Nakhjiri AT, Khakzad MJ, Rezayat SM, Ebrahimnejad P, Heydarinasab A, et al. Mesoporous silica nanoparticles as a versatile nanocarrier for cancer treatment: A review. J Mol Liq. 2021;328:115417.

Tandale P, Choudhary N, Singh J, Sharma A, Shukla A, Sriram P, et al. Fluorescent quantum dots: An insight on synthesis and potential biological application as drug carrier in cancer. Biochem Biophys Reports. 2021;26:100962.

Tang S, Yin Q, Su J, Sun H, Meng Q, Chen Y, et al. Inhibition of metastasis and growth of breast cancer by pH-sensitive poly (β-amino ester) nanoparticles co-delivering two siRNA and paclitaxel. Biomaterials. 2015; 48:1–15.

Taschauer A, Polzer W, Pöschl S, Metz S, Tepe N, Decker S, et al. Combined chemisorption and complexation generate siRNA nanocarriers with biophysics optimized for efficient gene knockdown and air–blood barrier crossing. ACS Appl Mater Interfaces. 2020;12: 30095–111.

Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid nanoparticles - from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. ACS Nano. 2021;15:16982–7015.

Torres-Vanegas JD, Cruz JC, Reyes LH. Delivery systems for nucleic acids and proteins: barriers, cell capture pathways and nanocarriers. Pharmaceutics. 2021;13(3):428.

Tran P, Park J-S. Recent trends of self-emulsifying drug delivery system for enhancing the oral bioavailability of poorly water-soluble drugs. J Pharm Investig. 2021;51:439–63.

Tran T, Rades T, Müllertz A. Formulation of self-nanoemulsifying drug delivery systems containing monoacyl phosphatidylcholine and Kolliphor® RH40 using experimental design. Asian J Pharm Sci. 2018; 13:536–45.

van den Berg AIS, Yun C-O, Schiffelers RM, Hennink WE. Polymeric delivery systems for nucleic acid therapeutics: Approaching the clinic. J Control Release. 2021;331:121–41.

van den Brand D, Mertens V, Massuger LFAG, Brock R. siRNA in ovarian cancer – Delivery strategies and targets for therapy. J Control Release. 2018;283:45–58.

Vasile C. Polymeric nanomaterials. In: Vasile C (ed): Polymeric nanomaterials in nanotherapeutics (pp 1-66). Amsterdam: Elsevier, 2019.

Wan Z, Zheng R, Moharil P, Liu Y, Chen J, Sun R, et al. Polymeric micelles in cancer immunotherapy. Molecules. 2021;26(5):1220.

Wang D, Xu X, Zhang K, Sun B, Wang L, Meng L, et al. Codelivery of doxorubicin and MDR1-siRNA by mesoporous silica nanoparticles-polymerpolyethylenimine to improve oral squamous carcinoma treatment. Int J Nanomedicine. 2017;13:187–98.

Wang F, Gao J, Xiao J, Du J. Dually gated polymersomes for gene delivery. Nano Lett. 2018;18: 5562–8.

Wang X, Zhang M, Woloshun RR, Yu Y, Lee JK, Flores SRL, et al. Oral administration of ginger-derived lipid nanoparticles and Dmt1 siRNA potentiates the effect of dietary iron restriction and mitigates pre-existing iron overload in Hamp KO mice. Nutrients. 2021; 13(5):1686.

Wen Y, Bai H, Zhu J, Song X, Tang G, Li J. A supramolecular platform for controlling and optimizing molecular architectures of siRNA targeted delivery vehicles. Sci Adv. 2020;6(31):eabc2148.

Witika BA, Makoni PA, Matafwali SK, Chabalenge B, Mwila C, Kalungia AC, et al. Biocompatibility of biomaterials for nanoencapsulation: Current Approaches. Nanomaterials. 2020;10(9):1649.

Xiong X-B, Lavasanifar A. Traceable multifunctional micellar nanocarriers for cancer-targeted co-delivery of MDR-1 siRNA and doxorubicin. ACS Nano. 2011; 5:5202–13.

Xu C, Yu B, Qi Y, Zhao N, Xu F. Versatile types of cyclodextrin ‐ based nucleic acid delivery systems. Adv Healthc Mater. 2021a;10(1):2001183.

Xu X, Xie K, Zhang X-Q, Pridgen EM, Park GY, Cui DS, et al. Enhancing tumor cell response to chemotherapy through nanoparticle-mediated codelivery of siRNA and cisplatin prodrug. Proc Natl Acad Sci. 2013;110:18638–43.

Xu Y, Michalowski CB, Beloqui A. Advances in lipid carriers for drug delivery to the gastrointestinal tract. Curr Opin Colloid Interface Sci. 2021b;52:101414.

Xue C, Hu S, Gao Z-H, Wang L, Luo M-X, Yu X, et al. Programmably tiling rigidified DNA brick on gold nanoparticle as multi-functional shell for cancer-targeted delivery of siRNAs. Nat Commun. 2021a;12(1): 2928.

Xue X, Li J, Fan Y, Shen M, Shi X. Gene silencing-mediated immune checkpoint blockade for tumor therapy boosted by dendrimer-entrapped gold nanoparticles. Sci China Mater. 2021b;64:2045–55.

Yan X, Yang Y, Sun Y. Dendrimer applications for cancer therapies. J Phys Conf Ser. 2021;1948(1): 012205.

Yao X, Huang P, Nie Z. Cyclodextrin-based polymer materials: From controlled synthesis to applications. Prog Polym Sci. 2019;93:1–35.

Yau A, Lee J, Chen Y. Nanomaterials for protein delivery in anticancer applications. Pharmaceutics. 2021;13(2):155.

Yonezawa S, Koide H, Asai T. Recent advances in siRNA delivery mediated by lipid-based nanoparticles. Adv Drug Deliv Rev. 2020;154-155:64-78.

Yu YH, Kim E, Park DE, Shim G, Lee S, Kim YB, et al. Cationic solid lipid nanoparticles for co-delivery of paclitaxel and siRNA. Eur J Pharm Biopharm. 2012; 80:268–73.

Yuan Z, Guo X, Wei M, Xu Y, Fang Z, Feng Y, et al. Novel fluorinated polycationic delivery of anti-VEGF siRNA for tumor therapy. NPG Asia Mater. 2020;12 (1):34.

Zatsepin TS, Kotelevtsev YV, Koteliansky V. Lipid nanoparticles for targeted siRNA delivery - going from bench to bedside. Int J Nanomedicine. 2016;11:3077-86.

Zheng C, Zheng M, Gong P, Deng J, Yi H, Zhang P, et al. Polypeptide cationic micelles mediated co-delivery of docetaxel and siRNA for synergistic tumor therapy. Biomaterials. 2013;34:3431–8.

Zheng M, Jiang T, Yang W, Zou Y, Wu H, Liu X, et al. The siRNAsome: a cation-free and versatile nanostructure for siRNA and drug co-delivery. Angew Chemie Int Ed. 2019;58:4938–42.

Zhu C, Jung S, Luo S, Meng F, Zhu X, Park TG, et al. Co-delivery of siRNA and paclitaxel into cancer cells by biodegradable cationic micelles based on PDMAEMA–PCL–PDMAEMA triblock copolymers. Biomaterials. 2010;31:2408–16.

Zhu L, Perche F, Wang T, Torchilin VP. Matrix metalloproteinase 2-sensitive multifunctional polymeric micelles for tumor-specific co-delivery of siRNA and hydrophobic drugs. Biomaterials. 2014;35:4213–22.

Zhuang Y, Cui W. Biomaterial-based delivery of nucleic acids for tissue regeneration. Adv Drug Deliv Rev. 2021;176:113885.

Zou M, Du Y, Liu R, Zheng Z, Xu J. Nanocarrier-delivered small interfering RNA for chemoresistant ovarian cancer therapy. Wiley Interdiscip Rev RNA. 2021; 12(5):e1648

Zou Y, Sun X, Wang Y, Yan C, Liu Y, Li J, et al. Single siRNA nanocapsules for effective siRNA brain delivery and glioblastoma treatment. Adv Mater. 2020; 32(24):2000416.

Zou Y, Zheng M, Yang W, Meng F, Miyata K, Kim HJ, et al. Virus-mimicking chimaeric polymersomes boost targeted cancer siRNA therapy in vivo. Adv Mater. 2017;29(42):1703285.

Zu M, Ma Y, Cannup B, Xie D, Jung Y, Zhang J, et al. Oral delivery of natural active small molecules by polymeric nanoparticles for the treatment of inflammatory bowel diseases. Adv Drug Deliv Rev. 2021;176: 113887.