Nonionic surfactant vesicles for delivery of RNAi therapeutics

RNAi is a promising potential therapeutic approach for many diseases. A major barrier to its clinical translation is the lack of efficient delivery systems for siRNA. Among nonviral vectors, nonionic surfactant vesicles (niosomes) have shown a great deal of promise in terms of their efficacy and toxicity profiles. Nonionic surfactants have been shown to be a superior alternative to phospholipids in several studies. There is a large selection of surfactants with various properties that have been incorporated into niosomes. Therefore, there is great potential for innovation in terms of nisome composition. This article summarizes recent advancements in niosome technology for the delivery of siRNA.

KEYWORDS: gene delivery ▪ niosome ▪ nonionic surfactant vesicle ▪ siRNA delivery

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Delivery of RNAi therapeutics

Gene silencing-based therapy has the potential to transform modern medicine [1,2]. Synthetic siRNAs or miRNA mimics can be incorporated into RNA-induced silencing complexes to knockdown target genes [3]. A related therapeutic strategy to inhibit miRNA function is by introduction of miR inhibitors, sometimes known as anti-miRs or antagomirs. It is worth noting that RNAi can be induced by gene transfer in the form of shRNAs, by either viral or nonviral vectors, which are then transcribed and processed into active miRNA duplexes by innate endonucleases drosha and dicer. However, this strategy requires nuclear delivery of a large DNA molecule, such as a plasmid, which faces increased challenges in delivery [2,4]. By contrast, synthetic RNAi agents are much smaller in size (duplexes of oligomers of approximately 21 bases in length or in the case of anti-miRs, short single-stranded oligomers) and can incorporate chemical modifications into the backbone and termini for improved stability [5]. Despite these advantages, oligonucleotide agents are high-molecular-weight polyanions that cannot readily diffuse across cellular membranes, which presents a significant barrier to delivery [6,7]. Moreover, these molecules need to resist degradation during systemic circulation, extravasate and get across the cellular membrane to reach the cytoplasmic site of therapeutic action. The most promising strategies for RNAi delivery seem to be a combination of oligonucleotide backbone chemical modifications and formulation into nanoparticles [8].

Nanoparticles for nucleic acid delivery are typically synthesized by a self-assembly process driven by electrostatic interactions between a cationic polymer or lipid and an anionic nucleic acid, such as siRNA. A net positive charge of the assembled particles can mediate cellular uptake via electrostatic adhesion to cellular surfaces, which carry a slight negative charge. This basis of charge interaction is very helpful in facilitating siRNA delivery *in vitro* [3–4]. However, *in vivo*, highly charged particles are rapidly cleared from the circulation due to strong interactions with plasma components and the reticuloendothelial system [9]. As a result, there is generally little correlation between optimal compositions for high delivery efficiency of a delivery vehicle *in vitro* and *in vivo* [10]. To optimize *in vivo* delivery, it is important to make stable nanoparticles that can survive circulation, but not so stable that they are rendered inactive following cellular internalization. Achieving this balance requires rational design of nanoparticle composition [11]. A well-known system for siRNA delivery is based on stable nucleic acid lipid particles, for example, with a composition of cholesterol, dipalmitoylphosphatidylcholine, 3-*N*-[(ω-methoxy poly(ethylene glycol)2000)carbamoyl]-1,2-dimyrestyloxypropylamine and the cationic component 1,2-dilinoleyloxy-3-*N*,*N*-dimethylaminopropane [12]. Stable nucleic acid lipid particles are currently in Phase I and II clinical trials for the delivery of siRNAs; ALN-VSP02 and TKM-PLK1, respectively. Lipid nanoparticles for siRNA delivery have been the subject of several recent review articles [13–15]. Since delivery is key to the successful clinical translation of siRNA therapeutics, the need for additional effort in this area of research is evident.

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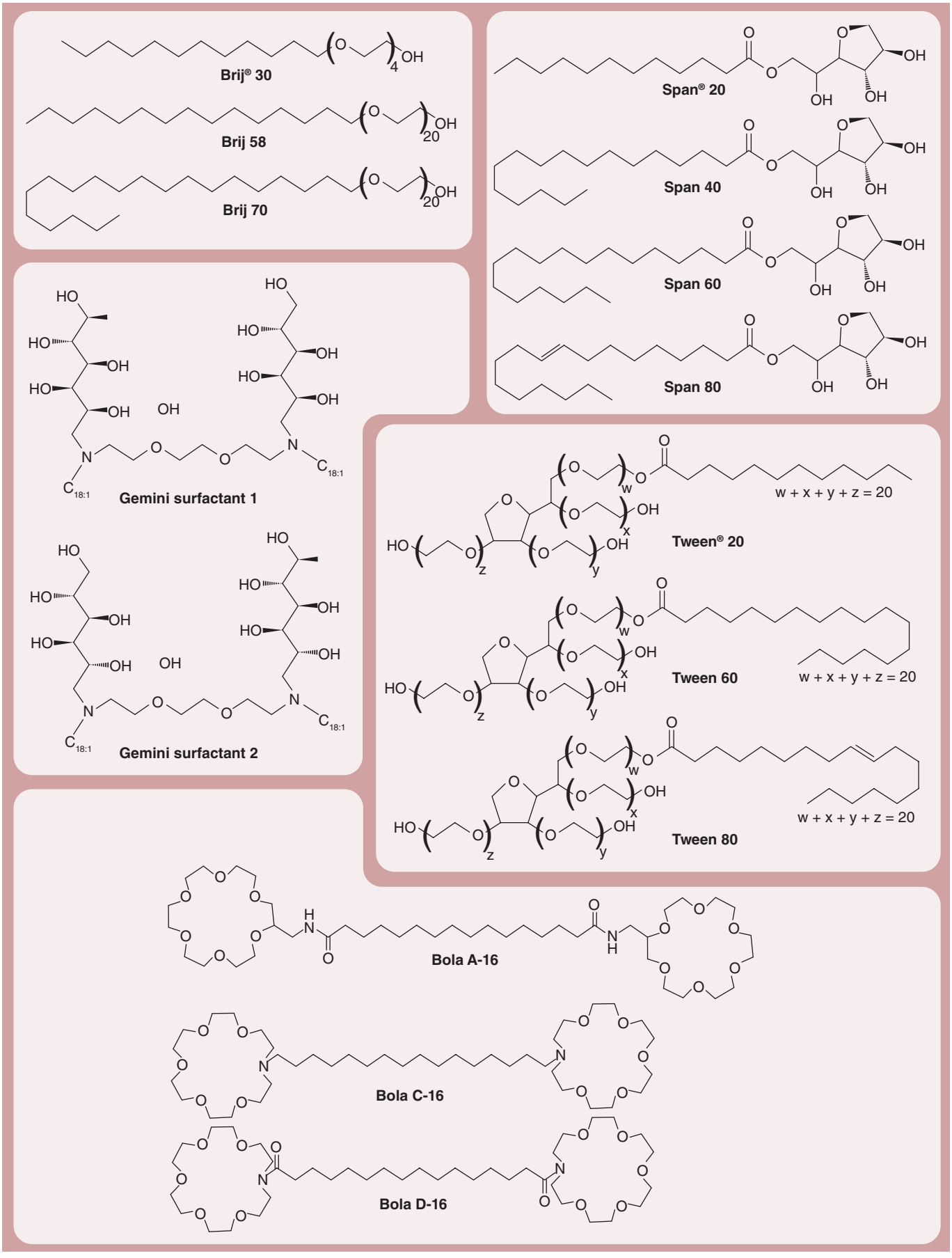
Brij® 30
Brij 58
Brij 70
Span® 20
Span 40
Span 60
Span 80
Gemini surfactant 1
Gemini surfactant 2
Tween® 20
Tween 60
Tween 80
w + x + y + z = 20
Bola A-16
Bola C-16
Bola D-16

Figure 1. Nonionic surfactant commonly used to prepare niosomes (facing page). Structures of alkyl ethers and alkyl glyceryl ethers (Brij®; Sigma Aldrich, MO, USA), sorbitan fatty acid esters (Span®; Sigma Aldrich), polyoxyethylene fatty acid esters (Tween®; Sigma Aldrich), Gemini surfactants 1 and 2, and Bola surfactants.

Nonionic surfactant vesicles for nucleic acid delivery

Similar to the zwitterionic phospholipids that form liposomes, nonionic surfactants with a cylindrical geometry are capable of forming bilayer vesicles, termed 'niosomes'. Like liposomes, these vesicles have been used for drug [16–31], gene [32–35] and siRNA [36,37] delivery. Niosomes and liposomes differ in several respects. First, surfactants are generally lower in cost and are potentially more stable than phospholipids, which are subject to oxidation and degradation by phospholipases. Indeed, niosomes may have longer shelf lives and be more stable than liposomes *in vivo*. The selection of synthetic surfactants may be further advantageous to lipids derived from natural sources, which can differ from batch to batch in terms of purity. A large selection of surfactants that display a wide range of properties desirable for specific drug delivery applications are readily available.

Composition of niosomes

Niosomes are typically composed of nonionic surfactants and cholesterol [38]. The ability to form bilayer vesicles from surfactants depends on their hydrophile–lipophile balance (HLB) value and critical packing parameter, as well as other factors. A critical packing parameter in the range of 0.5–1 indicates that a surfactant is likely to form spherical vesicles [39,40]. The optimal HLB value for high-loading efficiency niosomes is approximately 8.6. Surfactants with either higher or lower HLB values form vesicles with lower stability and lower volumes of entrapment [41–44]. The addition of cholesterol may be used to further increase the stability of niosomes.

Nonionic surfactants

Common nonionic surfactants that can be used to prepare niosomes are classified by chemical structure as described below (FIGURE 1) [38,39,45,46].

- Alkyl ethers and alkyl glyceryl ethers such as polyoxyethylene 4 lauryl ether (Brij® 30), polyoxyethylene cetyl ethers (Brij 58) and polyoxyethylene stearyl ethers (Brij 72 and Brij 76; Sigma Aldrich, MO, USA);
- Sorbitan fatty acid esters such as Span® 20, Span 40, Span 60 and Span 80 (Sigma Aldrich);
- Polyoxyethylene fatty acid esters (polysorbate) such as Tween® 20, Tween 60 and Tween 80 (Sigma Aldrich);
- Gemini surfactants [47,48];
- Bola Surfactants [49,50].

Cholesterol

Cholesterol may be combined with nonionic surfactants for the preparation of niosomes [51,52]. The gel-to-liquid phase transition of niosomes can be impeded by adding cholesterol, resulting in niosomes that are more rigid and less likely to lose the drug [39]. In a recent study, a 1:1 ratio of cholesterol and nonionic surfactants was shown to be optimal for producing physically stable niosomes [38], possibly owing to interactions between the surfactant and cholesterol [53].

Charge inducer components

Niosomes are often stabilized by the inclusion of a charged lipid. Examples of commonly used anionic lipids include dicetyl phosphate, dihexadecyl phosphate and lipoamine acid. For nucleic acid delivery, a cationic surfactant may be used so that the niosomes form electrostatic complexes with the negatively charged oligonucleotides [39,46]. A slight net surface charge of the nisome–oligonucleotide complexes leads to increased colloidal stability. However, excessive net charge can lead to rapid removal from systemic circulation by the reticuloendothelial system. Thus, a careful balancing of charge is essential to form an effective delivery vehicle.

Applications of niosomes

Niosomes constitute a versatile delivery platform that can be used in various pharmaceutical applications in addition to oligonucleotide delivery, as described in TABLE 1.

Niosomes as nucleic acid carriers

Like cationic liposomes, cationic niosomes can be used for nucleic acid delivery. Typical components of cationic niosomes include a nonionic surfactant, cholesterol and a cationic lipid [33,35]. Niosomes have been used successfully for topical gene delivery [46] and for synthesis of DNA vaccines [54,55]. In one study, plasmid delivery into rat skin mediated by niosomes resulted in high levels of β-galactosidase and luciferase reporter gene expression [56]. Manosroi *et al.*

Table 1. Pharmaceutical applications of niosomes.

Fields of application	Type of encapsulated drugs/agents	Ref.
Drug delivery	NSAIDs, anticancer, antibacterial, antifungal, antiviral, steroids, antiglaucoma, antidiabetics, local anesthetics, muscle relaxants, diagnostic agents, contraceptives, hormones, vitamins	[16–31]
Immunization	DNA vaccine, vaccine adjuvants	[54–61]
Protein/peptide drugs	Insulin, vasopressin	[38,53–64]
Nucleic acid delivery	Plasmid DNA, oligonucleotide	[32–35]
	siRNA	[36,37]

NSAID: Nonsteroidal anti-inflammatory drug.

demonstrated the use of elastic cationic niosomes composed of Tween 61 (Sigma Aldrich), cholesterol and DDAB as a topical delivery system for the tyrosinase gene as a treatment for vitiligo [57]. To improve the stability and cellular delivery of oligodeoxynucleotides (ODNs), Huang *et al.* prepared PEGylated cationic niosomes by modifying cationic niosomes with a PEG-conjugated lipid. Complexes of PEGylated cationic niosomes and ODNs showed a neutral ζ-potential with a particle size of approximately 300 nm. The PEG modification significantly decreased the serum binding and particle aggregation in the presence of serum, provided greater resistance to serum nuclease and enhanced the efficiency of ODN delivery [32]. Vyas *et al.* developed a niosome-based delivery system for DNA vaccines. DNA encoding hepatitis B surface antigen was encapsulated in niosomes composed of Span 85 (Sigma Aldrich) and cholesterol. The results showed that immunization using topical niosomes can elicit a comparable serum antibody titer and cytokine levels compared with those following intramuscular administration of recombinant hepatitis B surface antigen and topical administration of DNA vaccines in liposomes [54]. Gene transfer efficiency mediated by cationic niosomes is influenced by their composition, including the types of surfactants and cationic lipids used [32–35,58]. Paecharoenchai *et al.* investigated the effect of the structure of cationic lipids on pDNA transfection in HeLa cells mediated by cationic niosomes. The cationic niosomes were composed of Span 20, cholesterol and spermine derivative-based cationic lipids with varying acyl chain lengths (carbon [C]14, C16 and C18). The results showed that the transfection efficiency of the Span 20–niosomes was the highest for the spermine–C14 formulation following the order of: spermine–C14 > spermine–C16 > spermine–C18. In addition, Span 20–niosomes showed low cytotoxicity and hemolytic activity [35].

Niosomes as siRNA carriers

In addition to gene delivery, niosomes have been shown to facilitate siRNA delivery [36,37]. A general scheme for noisome-mediated siRNA delivery is shown in Figure 2. For example, a Span 80-based cationic niosome formulation, also known as SPANosome, was developed specifically for siRNA delivery. It contained 1,2-dioleoyl-3-trimethylammonium-propane as a cationic lipid and D-α-tocopheryl PEG-1000 succinate as a PEGylating lipid [36] at 1 or 5 n/n%. The particle size of empty SPANosomes was 25–40 nm, which decreased with increasing amounts of D-α-tocopheryl PEG-1000 succinate in the formulation. Optimization of the SPANosome–siRNA formulation was carried out by altering the siRNA/surfactant ratio. A decrease in this ratio resulted in smaller particle sizes and increased ζ-potential. The SPANosome–siRNA formulation showed colloidal stability for at least 3 weeks.

Gene silencing activity mediated by SPANosome

Gene silencing activity was evaluated in MDA-MB-231-green fluorescent protein (GFP) cells, which were stably transfected with GFP, using SPANosomes carrying the siRNA siGFP. GFP silencing was shown to be dose dependent and reached 66% with the optimized formulation, which was greater than the silencing activity of Lipofectamine® 2000 (Life Technologies, CA, USA). In another experiment, the aromatase gene silencing mediated by SPANosome–siRNA was investigated in SK-Br-3 cells with a siRNA targeting aromatase, siArom. The results based on aromatase assay showed approximately a 77% knockdown by SPANosome–siRNA and the efficiency was greater than that of lipofectamine–siRNA. No significant cytotoxicity in MDA-MB-231 cells was observed with the SPANosome–siRNA formulations at concentrations below 20 μg/ml (~100 nM) siRNA [36]. SPANosomes have yet to

be tested *in vivo* for siRNA delivery so their safety remains to be established [56–58].

Cellular uptake mechanism of SPANosome–siRNA

SPANosome–siRNA was shown to be internalized by tumor cells primarily through the caveolae-mediated pathway, which does not lead to lysosomal delivery and, thus, is less degradative. By contrast, the pathway used by lipofectamine–siRNA was primarily clathrin-mediated endocytosis [37]. Intracellular trafficking of SPANosome–siRNA was studied using molecular beacons as probes of cytoplasmic delivery [37]. The results showed that SPANosome–siRNA had a longer intracellular half-life and greater delivery of molecular beacons into the cytoplasm relative to cationic liposomes–siRNA. Since Span 80 is known to form nonbilayer cubic phases, it may promote the destabilization of the endosomal membrane and subsequently enhance cytosolic delivery of the molecular beacon. Additionally, Huang *et al.* reported that Spans enhanced transfection mediated by cationic liposomes. This effect might be due to the abilities of Span to destabilize an endosomal membrane and also to promote phase transition from the lamellar phase to inverted hexagonal phase, resulting in cytoplasmic release of DNA [59]. Therefore, nonionic surfactants, such as Span 80, can be considered as 'helper lipids' to cationic lipids with greater efficiency than conventional helper lipids such as 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine and cholesterol, which are less active in the presence of serum. Given the wide selection of nonionic surfactants commercially available, there is ample space for innovation and optimization of niosome formulations for siRNA delivery.

Some recent publications on niosomes as gene/siRNA carriers are listed in TABLE 2.

Conclusion

siRNA and other oligonucleotide-based therapeutics represent great opportunities for drug

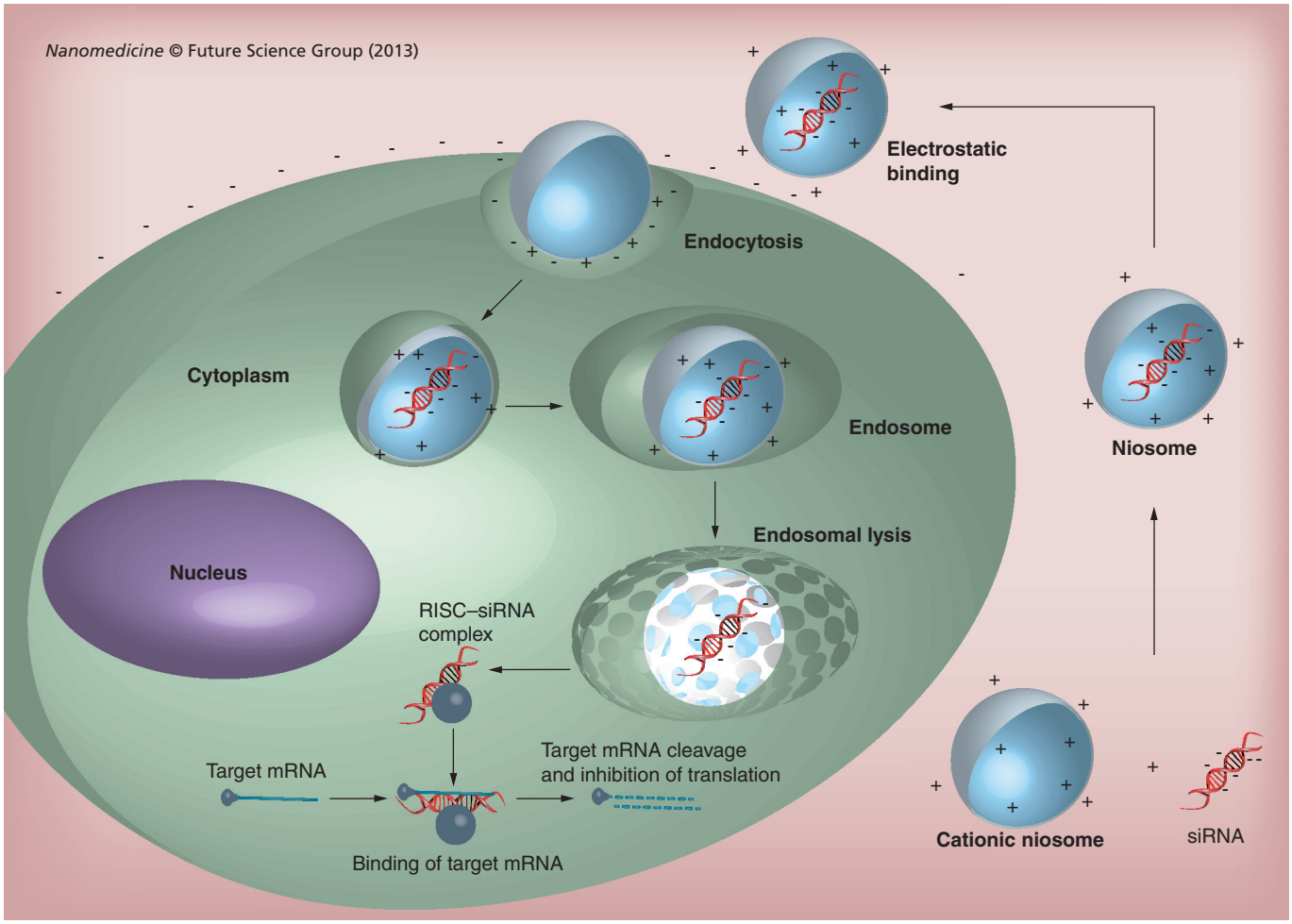

Figure 2. Niosome–siRNA delivery pathway. siRNA forms an electrostatic complex with the cationic niosome. Niosomes are then internalized by the cell and siRNA is released into the cytoplasm to associate with RISC. RISC degrades the sense strand of siRNA and recruits the target mRNA, which is subsequently degraded, thus inhibiting translation.

Table 2. Niosome-based gene/siRNA delivery systems.

Niosome compositions	DNA/siRNA	Model	Results	Ref.
Tween® 61 (Sigma Aldrich, MO, USA):Chol:DDAB (1:1:0.5 M ratio)	Tyrosinase plasmid (pMEL34)	Rat skin	Higher tyrosinase activity than the free plasmid	[57]
Tween 61:Chol:DDAB (1:1:0.5 M ratio)	Luciferase plasmid	Rat skin	Enhanced transdermal absorption of luciferase plasmid	[58]
Span® 20 (Sigma Aldrich):Chol:spermine–C14 Span 20:Chol:spermine–C16 Span 20:Chol:spermine–C18 (2.5:2.5:1 M ratio)	pEGFP-C2	HeLa cell line	High transfection efficiency with low cytotoxicity and low hemolytic effect; transfection efficiency is affected by cationic lipid structure	[35]
GDL:Chol:POE-10	β-galactosidase reporter	Rat skin	Intense staining of follicular and epidermal cells	[56]
Span 85 (Sigma Aldrich):Chol (7:3 M ratio)	HBsAg	BALB/c mice	High serum antibody titer and endogenous cytokines	[54]
Span 40 (Sigma Aldrich):DC-Chol (1:1 M ratio) with PEG2000-DSPE (5 mol%)	Oligodeoxynucleotides	COS-7 cell line	PEGylated cationic niosomes showed a higher efficiency of oligodeoxynucleotide cellular uptake and decreased the binding of serum protein	[32]
DOTAP:Span 80:TPGS (50:49:1/50:45:5 M ratio) SPANosomes	siGFP, siArom	MDA-MB-231 cells with/without stably transfected GFP, aromatase-expressing cell line SK-Br-3	Transfection efficiency greater than cationic liposome-based reagent, 66% GFP gene silencing, 77% aromatase gene silencing	[36]
DOTAP:Span 80:TPGS (50:49:1 M ratio) SPANosomes	FAM-siRNA, Cy3-MB, Silencer® renilla luciferase siRNA (Invitrogen, CA, USA)	SK Hep-1 cells with stable luciferase expression, (SK Hep-1 Luc) and flow cytometry	Divergent cellular pharmacokinetic profiles of the niosomes and liposomes were associated with different cellular entry pathways	[37]

*Chol: Cholesterol; DC-Chol: 1-cholesteryl 3-*N*-(dimethylaminoethyl) carbamate; DDAB: Dimethyl dioctadecyl ammonium bromide; DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane; FAM: Carboxyfluorescein; GDL: Glyceryl dilaurate; GFP: Green fluorescent protein; HBsAg: Hepatitis B surface antigen; PEG2000-DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-(PEG)-2000; POE-10: Polyoxyethylene-10 stearyl ether; siArom: siRNA targeting aromatase; siGFP: Silencer® enhanced GFP siRNA; Spermine–C14: N^1,N^1-dimyristeroyloxyethyl-spermine; Spermine-C16: N^1,N^1-palmitoyloxyethyl-spermine; Spermine–C18: N^1,N^1-steroyloxyethyl-spermine; TPGS: D-α-tocopheryl PEG-1000 succinate.*

development. Developing efficient delivery systems is the key to their successful clinical translation. Niosomes have shown superior activities over well-known lipid-based delivery systems. Careful selection of surfactant and lipid components determines the encapsulation, pharmacokinetic and release properties of niosomes. Like liposomes, niosomes may have applications in many pharmaceutical fields including conventional drug delivery, protein/peptide delivery, vaccine delivery and oligonucleotide delivery. Current data appear to suggest that the success of niosomes for siRNA delivery may be due to a combination of caveolae-mediated cellular entry and the membrane bilayer destabilization effect characteristic of surfactant molecules.

Future perspective

Niosome technology for the delivery of ODNs and siRNA is still in its early stages and there is much room for improvement and innovation. A large variety of surfactants and lipid combinations that could benefit the delivery system remain untested. Concerns relating to particle size and long-term colloidal stability will need to be addressed by careful adjustment of surface charge parameters and perhaps post-production considerations such as lyophilization. Determination of the *in vivo* efficacy of the formulation will be necessary moving forward to determine if off-target toxicity is a limiting factor for niosomes. Thus far, niosomes have only been tested *in vitro* or topically; demonstration of efficacy via paternal administration would further expand its application clinically. The application of targeting agents such as antibodies may also be of benefit to niosome formulations should off-target toxicity present an issue. Taken together, niosomes represent an exciting opportunity for the treatment of cancer and other diseases that do not respond well to traditional methods of treatment.

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Executive summary

Delivery of RNAi therapeutics

- The potential of RNAi therapeutics has been largely limited by inefficient methods of delivery.
- Nonviral vectors, which take advantage of electrostatic interactions with RNAi therapeutics, form stable complexes that promote delivery to the intracellular target.

Nonionic surfactant vesicles for nucleic acid delivery

- Niosomes possess a variety of chemical properties that make them advantageous relative to the classically used phospholipids.
- Niosomes are composed of nonionic surfactants, cholesterol and charge-inducer components.

Applications of niosomes

- Niosomes have shown success in the delivery of several classes of drug, including nucleic acid-based drugs.
- Among niosomes, SPANosomes, based on the surfactant Span® 80 (Sigma Aldrich, MO, USA), have experienced success owing to utilization of the caveolae-mediated pathway for cellular entry.

Conclusion

- The development of carrier systems is essential to the implementation of RNAi therapeutics.
- Niosomes demonstrate increased efficacy over conventional lipid-based delivery systems.

Future perspective

- Further optimization and characterization of niosome formulations will potentiate its activity and open doors for new treatment opportunities for patients.

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