ApoA1 and a small molecule drug. The drawback of these nanobiologics is that due to their hydrophobic core they do not allow the incorporation of polar structures such as nucleic acids, e.g. DNA and RNA.

Therefore, there is a need for improved delivery systems for therapeutic nucleic acids to the myeloid compartment.

SUMMARY

Present inventors were the first to develop a nanoparticle that allows delivery of a nucleic acid cargo to the myeloid compartment. More particularly, present inventors have developed stable, lipid-based nano-sized formulations (diameter ~10-200 nm) comprising an apolipoprotein and/or apolipoprotein mimetic, a phospholipid, a sterol, a cationic or ionizable cationic lipid and a nucleic acid, such as siRNA or mRNA. Without wishing to be bound by any theory, present inventors believe that the core of the nanoparticle comprises an assembly of nucleic acid interacting with the (ionizable) cationic lipid, wherein this core is packaged and buried within an outer protective surface or lipid shell comprising the apolipoprotein and/or an apolipoprotein mimetic, the phospholipid and the sterol, which functions as a surface barrier.

The nucleic acid is properly and stably incorporated into the nanoparticles of present disclosure, without the need of synthetic (non-natural) hydrophilic polymers or (lipid) conjugates of such polymers, such as polyethylene-glycol (PEG).

Furthermore, the nanoparticles of present disclosure also do not uncontrollably aggregate and/or coalesce, even when such synthetic (non-natural) hydrophilic polymers or (lipid) conjugates of such polymers are absent.

In addition, the nanoparticles of present disclosure have a targeting capability towards myeloid cells and other cells associated with the immune system, as a result of the presence of apolipoproteins and/or apolipoprotein mimetics at the outer surface of the nanoparticle.

Moreover, the nanoparticles as taught herein are stable, have a low toxicity or are non-toxic, have a high nucleic acid retention and a high nucleic acid activity.

Present inventors further have developed a controlled formulation process for successfully incorporating a nucleic acid in an apolipoprotein and/or apolipoprotein mimetic-based nanoparticle.

Accordingly, a first aspect of the disclosure provides a nanoparticle comprising:

a core surrounded by a surface layer, wherein:

the core comprises a nucleic acid and a cationic or ionizable cationic lipid; and

the surface layer comprises:

a phospholipid,

a sterol, and

an apolipoprotein or an apolipoprotein mimetic or a combination thereof.

The disclosure, in another aspect, relates to a composition comprising the nanoparticle as defined herein and a physiologically acceptable carrier.

The disclosure, in another aspect, relates to the nanoparticle as defined herein or the composition as defined herein for use as a medicament.

The disclosure, in another aspect, relates to the nanoparticle as defined herein or the composition as defined herein for use in the treatment of a disease by stimulating or inhibiting an innate immune response.

The disclosure further relates to a method for producing a nanoparticle, comprising the step of:

a) rapid mixing of lipid components in organic solvent with a nucleic acid in an aqueous buffer to produce lipid nanoparticles, wherein the lipid components comprise a phospholipid, a sterol, a cationic lipid or ionizable cationic lipid, and optionally a filler material, preferably a triglyceride, at a pH of 5.0 or lower; and

b) rapid mixing of the lipid nanoparticles (as prepared under a)) with an apolipoprotein, an apolipoprotein mimetic, or a combination thereof to produce the nanoparticle at a pH between 6.0 and 8.0.

The disclosure, in another aspect, relates to a method for producing a nanoparticle, comprising the step of:

a) rapid mixing of lipid components in organic solvent with a nucleic acid in an aqueous buffer to produce lipid nanoparticles, wherein the lipid components comprise a phospholipid, a sterol, a cationic lipid or ionizable cationic lipid, wherein the aqueous buffer has a pH of 5.0 or lower; and

b) rapid mixing of the lipid nanoparticles with an apolipoprotein, an apolipoprotein mimetic, or a combination thereof to produce the nanoparticle at a pH between 6.0 and 8.0.

The disclosure further relates to an *in vitro* or *ex vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the nanoparticle or the composition according to the disclosure with a cell.

The disclosure further relates to the nanoparticle according to the disclosure obtainable or obtained by the method according to the disclosure.

The disclosure further relates to an *in vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the nanoparticle or the composition according to the disclosure with a cell.

The disclosure further relates to the nanoparticle or the composition according to the disclosure for use in the *in vivo* delivery of a nucleic acid to a subject.

The disclosure further relates to a method for the *in vivo* delivery of a nucleic acid, the method comprising administering the nanoparticle or the composition according to the disclosure to a subject.

The disclosure further relates to a method for treating a disease or disorder in a subject in need thereof by stimulating or inhibiting an innate immune response, the method comprising administering a therapeutically effective amount of the nanoparticle or the composition according to the disclosure to the subject.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1: Schematic overview of apolipoprotein lipid nanoparticle (aNP) platform technology according to certain embodiments of the invention, for nucleic acid delivery. Without wishing to be bound by any theory, such RNA-aNPs are believed to be composed of a hydrophobic core containing optional filler material (e.g. triglycerides) and nucleic acids, such as RNA, complexed by (ionizable) cationic lipids. The hydrophobic core is enclosed and shielded by a surface layer or barrier, possibly a monolayer, containing phospholipids and sterols. The lipid nanoparticle's surface also comprises apolipoproteins for structural integrity, to prevent aggregation, to provide particle stability, to provide natural stealth and/or to facilitate interactions with immune cells.

Fig. 2: Schematic overview of an illustrative method according to certain embodiments of the invention for producing apolipoprotein lipid nanoparticles (aNP) containing nucleic acids such as RNA as described herein.

Fig. 3: siRNA retention in apolipoprotein nanoparticles (aNPs) according to certain embodiments of the invention and instability of comparative example nanoparticles (NPs) without apolipoprotein. (A) Representative aNP containing siRNA (siRNA-aNP) 18 and 34 were prepared according to the production procedure depicted in Figure 2 (white bars). Additionally, comparative NPs were prepared by omitting the procedure's second step, whereby apolipoprotein A1 is incorporated in the formulation (black bars). RNA retention was determined using the Ribogreen assay one day post formulating. (B) Representative image of the comparative example siRNA-NP formulation 18 that has no apolipoprotein A1 incorporated. (C) Representative cryogenic transmission electron micrographs of the comparative example siRNA-NP formulation 18 (scale bar 50 nm).

Fig. 4: The lipid composition of apolipoprotein lipid nanoparticles (aNP) containing siRNA (siRNA-aNP) according to certain embodiments of the invention influences their physicochemical properties and can be optimized to obtain siRNA-aNP with optimal characteristics. (A) One day after formulating, the library's individual siRNA-aNP

CLAIMS

1. A nanoparticle comprising a core surrounded by a surface layer, wherein:

the core comprises a nucleic acid and a cationic or ionizable cationic lipid; and

the surface layer comprises:

a phospholipid,

a sterol, and

an apolipoprotein or an apolipoprotein mimetic or a combination thereof.

2. The nanoparticle according to claim 1, wherein the apolipoprotein, apolipoprotein mimetic, or the combination thereof is located on the outer surface of the surface layer.

3. The nanoparticle according to claim 1 or 2, wherein the nanoparticle core further comprises a filler, preferably a filler selected from a triacylglyceride and a cholesterol acyl ester or combinations thereof.

4. The nanoparticle according to claim 3, wherein the triacylglyceride is tricaprylin and/or wherein the cholesterol acyl ester is cholesteryl caprylate and/or cholesteryl oleate.

5. The nanoparticle according to any one of the preceding claims, wherein the nucleic acid is RNA, DNA or a nucleic acid analogue.

6. The nanoparticle according to claim 5, wherein the RNA is microRNA (miRNA), small interfering RNA (siRNA), piwi-interacting RNA (piRNA), small nuclear RNA (snoRNA), transfer RNA (tRNA), tRNA-derived small RNA (tsRNA), small regulatory RNA (srRNA), messenger RNA (mRNA), modified mRNA, ribosomal RNA (rRNA), long non-coding RNA (lncRNA)or guide RNA (gRNA) or combinations thereof and/or modifications thereof.

7. The nanoparticle according to claim 5, wherein the DNA is single stranded or double stranded DNA.

8. The nanoparticle according to any one of claims 1 to 5, wherein the nucleic acid is an antisense oligonucleotide and the antisense oligonucleotide is single strand DNA or RNA consisting of nucleotide or nucleoside analogues containing modifications of the phosphodiester backbone or the 2' ribose.

9. The nanoparticle according to claim 8, wherein the nucleotide or nucleoside analogues are selected from locked nucleic acid (LNA), bridged nucleic acid (BNA), morpholino or peptide nucleic acid (PNA).

10. The nanoparticle according to any one of the preceding claims, wherein the apolipoprotein is selected from ApoA1, ApoA1-Milano, ApoA2, ApoA4, ApoA5, ApoB48,

ApoB100, ApoC-I, ApoC-II, ApoC-III, ApoC-IV, ApoD, ApoE, ApoF, ApoH, ApoL, ApoM, and combinations thereof.

11. The nanoparticle according to claim 10, wherein the apolipoprotein is selected from ApoA1, ApoA2, ApoA4, ApoA5, ApoB100, ApoC-I, ApoC-II, ApoC-III, ApoC-IV, ApoE, and combinations thereof.

12. The nanoparticle according to claim 10, wherein the apolipoprotein is selected from ApoA1, ApoA4, ApoA5, ApoB100, ApoC-III, ApoE, and combinations thereof.

13. The nanoparticle according to claim 10, wherein the apolipoprotein is selected from ApoA1, ApoB100, ApoE, and combinations thereof.

14. The nanoparticle according to any one of claims 1 to 13, wherein the apolipoprotein in the nanoparticle is used to:

- prevent aggregation upon preparation and storage;

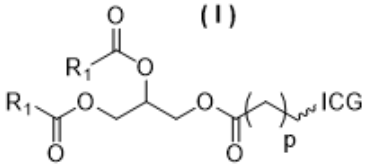
- improve in vivo stability;

- provide natural stealth; and/or

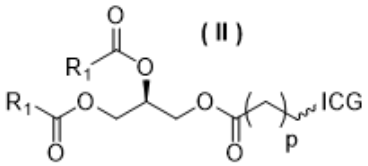
- facilitate interactions with immune cells.

15. The nanoparticle according to any one of the preceding claims, wherein the cationic or ionizable cationic lipid is selected from an ionizable cationic ester of a long chain alcohol, an ionizable cationic ester of a diglyceride or an ionizable cationic ester of a sterol or combinations thereof.

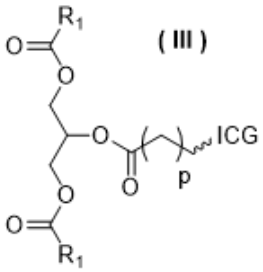
16. The nanoparticle according to any one of the preceding claims, wherein the ionizable cationic lipid is a molecule according to any one of Formulae (I), (II), (III), (IV) or (V)



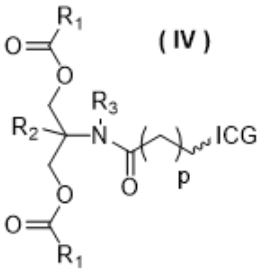
(I)



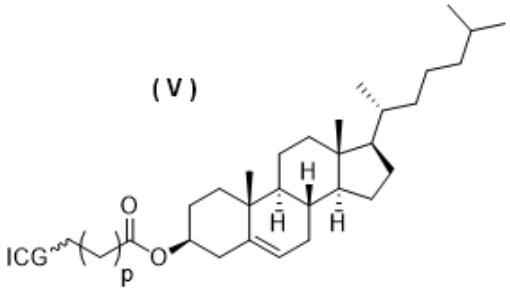
(II)



(III)

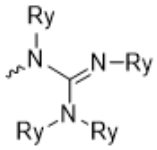


(IV)



(V)





wherein ICG is or , wherein the wavy line indicates the point of attachment to the compound of formulae (I), (II), (III), (IV) or (V);

p is an integer selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11;

each R_1 is independently selected from the group consisting of linear or branched C1-C19 alkyl, linear or branched C1-C19 alkenyl, aryl, arylene-alkyl and alkylene-aryl group, wherein said alkyl or alkenyl group optionally contains up to 5 heteroatoms, independently selected from O and N;

R_2 is selected from the group consisting of hydrogen, methyl, ethyl and a $-CH_2-O-C(O)-R_{1a}$;

R_3 is selected from the group consisting of hydrogen, aryl, arylene-alkyl, alkylene-aryl and linear C1-C6 alkyl group;

R_{1a} is selected from the group consisting of linear or branched C1-C19 alkyl, linear or branched C1-C19 alkenyl, aryl, arylene-alkyl and alkylene-aryl group, wherein said alkyl or alkenyl group optionally contains up to 5 heteroatoms, independently selected from O and N;

each R_x is independently selected from the group consisting of methyl, ethyl, propyl and $-CH_2-CH_2-OH$;

each R_y group is independently selected from the group consisting of hydrogen, linear or branched C1-C18 alkyl, aryl, arylene-alkyl or alkylene-aryl group, wherein said alkyl group optionally contains up to 5 heteroatoms, independently selected from O and N;

or rotamers, tautomers stereoisomers or regioisomers thereof.

17. The nanoparticle according to any one of the preceding claims, wherein the sterol is selected from cholesterol, desmosterol, stigmasterol, β-sitosterol, ergosterol, hopanoids, hydroxysteroid, phytosterol, steroids, hydrogenated cholesterol, campesterol, zoosterol, or combinations thereof.

18. The nanoparticle according to any one of the preceding claims, wherein:

the phospholipid is selected from a phosphatidylcholine (PC), a phosphatidylethanolamine (PE), a phosphatidylserine and a phosphatidylglycerol or combinations thereof.

19. The nanoparticle according to claim 18, wherein at least one, more preferably both, of the acyl groups in the phospholipid are long chain fatty acids,

20. The nanoparticle according to claim 19, wherein said long chain fatty acids are selected from myristoleic acid, palmitoleic acid and oleic acid or combinations thereof.

21. The nanoparticle according to any one of the preceding claims, wherein the phospholipid is selected from the group consisting of dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dilauroylphosphatidylglycerol (DLPG), dimyristoylphosphatidylglycerol (DMPG), dipalmitoylphosphatidylglycerol (DPPG), distearoylphosphatidylglycerol (DSPG), dioleoylphosphatidylglycerol (DOPG), dilauroyl phosphatidylethanolamine (DLPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), distearoyl phosphatidylethanolamine (DSPE), dilauroyl phosphatidylserine (DLPS), dimyristoyl phosphatidylserine (DMPS), dipalmitoyl phosphatidylserine (DPPS), distearoyl phosphatidylserine (DSPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), or combinations thereof.

22. The nanoparticle according to any one of the preceding claims, wherein:

the amount of apolipoprotein ranges from 0.08 to 2.0 mol%, such as from 0.10 to 2.0 mol%; and/or

the amount of phospholipid ranges from 5 to 90 mol%, such as from 15 to 90 mol%; and/or

the amount of sterol ranges from 2.5 to 65 mol%, such as from 2.5 to 50 mol%; and/or

the amount of cationic or ionizable cationic lipid ranges from 5.0 to 80 mol%, such as from 8.0 to 80 mol%, wherein the molar percentage is based solely on the combined amounts of the apolipoprotein, phospholipids, sterols and cationic or ionizable cationic lipids in the nanoparticle.

23. The nanoparticle according to any one of the preceding claims, wherein
the amount of apolipoprotein and/or apolipoprotein mimetic ranges from 0.1 to 90 weight%;
the amount of nucleic acid ranges from 0.01 to 90 weight%;
the amount of phospholipid ranges from 0.1 to 95 weight%;
the amount of sterol ranges from 0.1 to 95 weight%; and/or
the amount of cationic and/or ionizable cationic lipid ranges from 0.1 to 95 weight%,
wherein these weight percentages are based on the combined amounts of the apolipoprotein and/or apolipoprotein mimetic, the nucleic acid, the phospholipid, the sterol and the cationic and/or ionizable cationic lipid.

24. The nanoparticle according to any one of the preceding claims, wherein the ratio of apolipoprotein to phospholipid based on percentage molar weight is between 1:25 and 1:400, more preferably between 1:50 and 1:200, even more preferably between 1:75 and 1:150.

25. The nanoparticle according to any one of the preceding claims, wherein the ratio of apolipoprotein to phospholipid based on weight is from 2:1 to 1:10, more preferably from 1:1 to 1:5, even more preferably from 1:1.5 to 1:4.

26. The nanoparticle according to any one of the preceding claims having an average size of 10 to 100 nm, such as from 30 to 100 nm.

27. A composition comprising the nanoparticle according to any one of claim 1 to 26 and a physiologically acceptable carrier.

28. The composition according to claim 27, wherein the composition is a pharmaceutical composition.

29. The nanoparticle according to any one of claims 1 to 26, or the composition according to claim 27 or 28 for use as a medicament.

30. The nanoparticle according to any one of claims 1 to 26, or the composition according to claim 27 or 28 for use in the treatment of a disease by stimulating or inhibiting an innate immune response.

31. The nanoparticle or composition for use according to claim 30, wherein said disease is a cancer, a cardiovascular disease, an autoimmune disorder or xenograft rejection.

32. Method for producing a nanoparticle, comprising the step of:

a) rapid mixing of lipid components in organic solvent with a nucleic acid in an aqueous buffer to produce lipid nanoparticles, wherein the lipid components comprise a phospholipid, a sterol, a cationic lipid or ionizable cationic lipid, wherein the aqueous buffer has a pH of 5.0 or lower; and

b) rapid mixing of the lipid nanoparticles with an apolipoprotein, an apolipoprotein mimetic, or a combination thereof to produce the nanoparticle at a pH between 6.0 and 8.0.

33. The nanoparticle according to any one of claim 1 to 26, wherein the nanoparticle is obtainable or obtained by the method of claim 32.

34. An *in vitro* or *ex vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the nanoparticle according to any one of claims 1 to 26 or the composition according to claim 27 or 28 with a cell.

35. The nanoparticle according to any one of claims 1 to 26 or the composition according to claim 27 or 28 for use in the *in vivo* delivery of a nucleic acid to a subject, optionally to the myeloid compartment or the spleen of said subject.