lipid formulation of mRNA can clog a nebulizer during nebulization and that the formulation loses efficacy upon nebulization.

Accordingly, the task underlying the present invention was providing a composition comprising a nucleic acid, which composition is suitable for delivering a nucleic acid to a cell in the respiratory tract in a subject upon nebulization and inhalation of said composition in a manner that the nucleic acid efficiently exerts its intended function following the inhalation, such as leading to the production of a protein in a lung cell, leading to the downregulation or upregulation of expression of an endogenous gene or leading to gene repair. Moreover, it should be possible to nebulize an effective dose of the composition within a reasonable period of time, and the components of the composition should remain intact during nebulization.

In the context of the present invention, it was found that a suspension formulation of lipid or lipidoid nanoarticles (LNPs) which comprise a nucleic acid and an ionizable lipid or an ionizable lipidoid, and which are suspended in an aqueous vehicle solution comprising a poly(ethylene oxide)-poly(propylene oxide) block copolymer can be efficiently nebulized while a negative impact of the nebulization procedure on the integrity of the nanoparticles and the nucleic acid contained therein can be prevented. In particular, the use of this formulation for the preparation of an aerosol allows a significantly improved resistance of the particles against aggregation during the nebulization process and a beneficial retention of the transfection efficiency of the nucleic acid after the nebulization process to be achieved.

A summary of the ~~aspects of the invention~~ disclosure is provided in the following items.

1. An aqueous suspension formulation for aerosol formation, said suspension formulation comprising lipid or lipidoid nanoparticles which are suspended in an aqueous vehicle solution, wherein the lipid or lipidoid nanoparticles comprise the following components (a) and (b):

   (a) a nucleic acid and

   (b) an ionizable lipid or an ionizable lipidoid;

and wherein the aqueous vehicle solution comprises a triblock copolymer which contains one poly(propylene oxide) block and two poly(ethylene oxide) blocks.

2. The suspension formulation in accordance with item 1, wherein the nucleic acid is selected from RNA and plasmid DNA.

3. The suspension formulation in accordance with item 1 or 2, wherein the nucleic acid is selected from mRNA, siRNA, miRNA, antisense RNA, tRNA, and noncoding RNA and is more preferably mRNA.

67. The aerosol in accordance with any of items 33 to 60 for use in the treatment or prevention of a disease or disorder via a nucleic acid-based therapy, wherein the treatment or prevention comprises the administration of the aerosol to or via the respiratory tract of a subject, preferably via pulmonary administration or nasal administration.

68. The aqueous suspension formulation for use in accordance with item 66 or the aerosol for use in accordance with item 67, wherein the disease or disorder to be treated or prevented is a pulmonary disease.

69. A method of treatment, comprising the nebulization of the aqueous suspension formulation in accordance with any of items 1 to 28 and 30 and the administration of the aerosol provided by nebulization to or via the respiratory tract of a subject, preferably via pulmonary administration or nasal administration.

70. A method of treatment, comprising the administration of the aerosol in accordance with any of items 33 to 60 to or via the respiratory tract of a subject, preferably via pulmonary administration or nasal administration.

71. The method in accordance with item 69 or 70 for the treatment of a pulmonary disease.

In one aspect, the present disclosure provides an aqueous suspension formulation for aerosol formation, said suspension formulation comprising lipid or lipidoid nanoparticles which are suspended in an aqueous vehicle solution,
wherein the lipid or lipidoid nanoparticles comprise the following components (a) and (b):

(a) a nucleic acid and

(b) an ionizable lipid or an ionizable lipidoid;

and wherein the aqueous vehicle solution comprises a triblock copolymer which contains one poly(propylene oxide) block and two poly(ethylene oxide) blocks.

In another aspect, the present disclosure provides a nebulizer, which comprises a compartment wherein the aqueous suspension formulation for aerosol formation as disclosed herein is contained.

In another aspect, the present disclosure provides an aerosol comprising aerosol droplets dispersed in a gas phase, wherein the aerosol droplets comprise lipid or lipidoid nanoparticles and an aqueous vehicle solution for the nanoparticles,
wherein the lipid or lipidoid nanoparticles comprise the following components (a) and (b):

(a) a nucleic acid and

(b) an ionizable lipid or an ionizable lipidoid;

and wherein the aqueous vehicle solution comprises a triblock copolymer which contains one poly(propylene oxide) block and two poly(ethylene oxide) blocks.

In another aspect, the present disclosure provides an aqueous suspension formulation as disclosed herein for use as a medicament, wherein the aqueous suspension formulation is to be nebulized and the aerosol provided by nebulization is to be administered to a subject, preferably wherein an effective dose of the nucleic acid is delivered by nebulization within 60 minutes or less, more preferably 30 minutes or less.

In another aspect, the present disclosure provides an aqueous suspension formulation as disclosed herein for use in the treatment or prevention of a disease or disorder via a nucleic acid-based therapy, wherein the treatment or prevention comprises the nebulization of the aqueous suspension formulation and the administration of the aerosol provided by nebulization to or via the respiratory tract of a subject, preferably via pulmonary administration or nasal administration.

In another aspect, the present disclosure provides use of the aqueous suspension formulation as disclosed herein in the manufacture of a medicament for the treatment or prevention of a pulmonary disease or disorder via a nucleic acid-based therapy, wherein the treatment or prevention comprises the nebulization of the aqueous suspension formulation and the administration of the aerosol provided by nebulization to or via the respiratory tract of a subject, preferably via pulmonary administration or nasal administration.

It will be understood that the summary in the above items forms a part of the general disclosure of the present ~~invention~~disclosure, such that the information provided in the following detailed description, e.g. with regard to further preferred embodiments or optional features, also applies for the above items and *vice versa*.

In the following, a detailed description of the invention will be provided. As will be understood by the skilled person, and unless indicated otherwise in a specific context, the information provided in this context applies to all aspects of the invention, including the aqueous suspension formulation for aerosol formation in accordance with the invention (which may be referred to herein as the “aqueous suspension formulation” or simply as “suspension formulation”), the aerosol in accordance with the invention, and methods and uses involving the suspension formulation or the aerosol.

First, the nanoparticles and their components will be explained. The aqueous suspension formulation for aerosol formation and the aerosol in accordance with the invention comprise lipid nanoparticles or lipidoid nanoparticles. Thus, unless specifically indicated to the contrary, a reference to “nanoparticles” or to “LNPs” herein encompasses the lipid nanoparticles as well as the lipidoid nanoparticles. Moreover, since the aerosol in accordance with the invention can

Claims

1. An aqueous suspension formulation for aerosol formation, said suspension formulation comprising lipid or lipidoid nanoparticles which are suspended in an aqueous vehicle solution, wherein the lipid or lipidoid nanoparticles comprise the following components (a) and (b):

(a) a nucleic acid and

(b) an ionizable lipid or an ionizable lipidoid;

and wherein the aqueous vehicle solution comprises a triblock copolymer which contains one poly(propylene oxide) block and two poly(ethylene oxide) blocks.

2. The suspension formulation in accordance with claim 1, wherein the concentration of the nucleic acid in the suspension formulation ranges from 0.01 to 10 mg/mL, based on the total volume of the suspension formulation.

3. The suspension formulation in accordance with claim 1 or 2, wherein the nanoparticles have a Z-average diameter, as determined by dynamic light scattering, in the range of 10 to 500 nm.

4. The suspension formulation in accordance with any of claims 1 to 3, wherein the nanoparticles further comprise one or more of the following components (c1) to (c6):

(c1) a non-ionizable lipid having a sterol structure;

(c2) a phosphoglyceride lipid;

(c3) a PEG-conjugated lipid;

(c4) a polysarcosine-conjugated lipid

(c5) a PASylated lipid; and

(c6) a cationic polymer.

5. The suspension formulation in accordance with any of claims 1 to 4, wherein the nanoparticles comprise:

30 to 65 mol% of the ionizable lipid or ionizable lipidoid (b), and one or more of the following components:

10 to 50 mol% of the lipid having a sterol structure (c1),

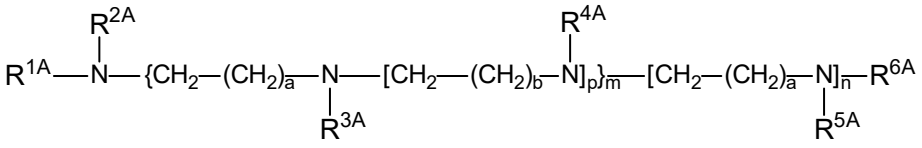
4 to 50 mol% of the phosphoglyceride lipid (c2),

0.5 to 10 mol% of one of the PEG-conjugated lipid (c3), the polysarcosine-conjugated lipid (c4) and the PASylated lipid (c5), or of any combination thereof,

0.5 to 10 mol% of the cationic polymer (c6),

such that the sum of (b) and (c1) to (c6) amounts to 100 mol%.

6. The suspension formulation in accordance with any of claims 1 to 5, wherein the nanoparticles comprise an ionizable lipidoid (b) of the following formula (b-1),



(b-1)

wherein:

a is 1 and b is an integer of 2 to 4; or a is an integer of 2 to 4 and b is 1,

p is 1 or 2,

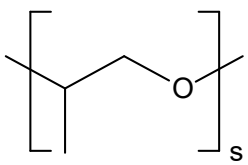
m is 1 or 2; n is 0 or 1 and m+n is ≥ 2; and

R^{1A} to R^{6A} are independently of each other selected from: hydrogen; $-CH_2-CH(OH)-R^{7A}$, $-CH(R^{7A})-CH_2-OH$, $-CH_2-CH_2-(C=O)-O-R^{7A}$, $-CH_2-CH_2-(C=O)-NH-R^{7A}$; $-CH_2-R^{7A}$; $-C(NH)-NH_2$; a poly(ethylene glycol) chain; and a receptor ligand; wherein R^{7A} is selected from C3-C18 alkyl and C3-C18 alkenyl having one C-C double bond;

provided that at least two residues among R^{1A} to R^{6A} are selected from $-CH_2-CH(OH)-R^{7A}$, $-CH(R^{7A})-CH_2-OH$, $-CH_2-CH_2-(C=O)-O-R^{7A}$, $-CH_2-CH_2-(C=O)-NH-R^{7A}$ and $-CH_2-R^{7A}$, wherein R^{7A} is selected from C3-C18 alkyl or C3-C18 alkenyl having one C-C double bond;

or a protonated form thereof, wherein one or more of the nitrogen atoms contained in the compound of formula (b-1) are protonated to provide a compound carrying a positive charge.

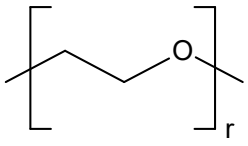
7. The suspension formulation in accordance with any of claims 1 to 6, wherein the triblock copolymer is an A-B-A triblock copolymer which contains one poly(propylene oxide) block B of formula (p-1):



(p-1)

wherein s is an integer of 15 to 67, preferably 20 to 40 and

two poly(ethylene oxides) blocks A of formula (p-2):



(p-2)

wherein r is, independently for each block, an integer of 2 to 130, preferably 50 to 100, and more preferably 60 to 90.

8. The suspension formulation in accordance with any of claims 1 to 7, which comprises the triblock copolymer at a concentration of 0.05 to 5 % (w/v, at a temperature of 25 °C), preferably 0.1 to 2%, based on the total volume of the suspension formulation.

9. The aqueous suspension formulation for aerosol formation according to any of claims 1 to 8, wherein the nucleic acid is ribonucleic acid (RNA), more preferably single stranded RNA, most preferably mRNA, optionally the RNA is coding or non-coding RNA, preferably single-stranded coding or noncoding RNA, preferably the noncoding RNA is not siRNA.

~~9.~~10. A nebulizer, which comprises a compartment wherein the aqueous suspension formulation for aerosol formation in accordance with any of claims 1 to ~~8~~ 9 is contained.

~~10.~~11. An aerosol comprising aerosol droplets dispersed in a gas phase, wherein the aerosol droplets comprise lipid or lipidoid nanoparticles and an aqueous vehicle solution for the nanoparticles,

wherein the lipid or lipidoid nanoparticles comprise the following components (a) and (b):

(a) a nucleic acid and

(b) an ionizable lipid or an ionizable lipidoid;

and wherein the aqueous vehicle solution comprises a triblock copolymer which contains one poly(propylene oxide) block and two poly(ethylene oxide) blocks.

~~11.~~12. The aerosol in accordance with claim ~~10~~11, wherein the mass median aerodynamic diameter (MMAD) of the aerosol droplets ranges from 2 to 10 µm.

~~12.~~13. The aerosol in accordance with claim 11 or 12, wherein the nanoparticles have a Z-average diameter, as determined by dynamic light scattering, in the range of 10 to 500 nm, more preferably in the range of 10 to 250 nm, still more preferably 20 to 200 nm.

~~13.~~14. The aerosol in accordance with any of claims ~~10~~ 11 to ~~12~~13, which is obtainable by nebulization of an aqueous suspension formulation in accordance with any of claims 1 to ~~8~~9.

~~14.~~15. ~~The~~ An aqueous suspension formulation in accordance with any of claims 1 to ~~8~~ 9 for use as a medicament, wherein the aqueous suspension formulation is to be nebulized and the aerosol provided by nebulization is to be administered to a subject, preferably wherein an effective dose of the nucleic acid is delivered by nebulization within 60 minutes or less, more preferably 30 minutes or less.

~~15.~~16. ~~The~~ An aqueous suspension formulation in accordance with any of claims 1 to ~~8~~ 9 for use in the treatment or prevention of a disease or disorder via a nucleic acid-based therapy, wherein the treatment or prevention comprises the nebulization of the aqueous suspension formulation and the administration of the aerosol provided by nebulization to or via the respiratory tract of a subject, preferably via pulmonary administration or nasal administration.

~~16.~~17. The aqueous suspension formulation for use in accordance with claim ~~15~~16, wherein the disease or disorder to be treated or prevented is a pulmonary disease.

18. Use of the aqueous suspension formulation in accordance with any of claims 1 to 9 in the manufacture of a medicament for the treatment or prevention of a pulmonary disease or disorder via a nucleic acid-based therapy, wherein the treatment or prevention comprises the nebulization of the aqueous suspension formulation and the administration of the aerosol provided by nebulization to or via the respiratory tract of a subject, preferably via pulmonary administration or nasal administration.