Thus, there remains a need in the art for efficient methods and compositions for introducing RNA into cells which avoid the disadvantages accompanied by use of PEG. The present disclosure addresses these and other needs.

The inventors surprisingly found that the RNA particle formulations described herein fulfill the above mentioned requirements. In particular it is demonstrated that polysarcosine-lipid conjugates are suitable components for assembly of RNA nanoparticles. Polysarcosine is composed of repeated units of the natural amino acid sarcosine (*N*-methylglycine) and is biodegradable. Polysarcosine-lipid conjugates enable manufacturing of RNA nanoparticles with different techniques, resulting in defined surface properties and controlled size ranges. Manufacturing can be done by robust processes, compliant with the requirements for pharmaceutical manufacturing. The particles can be end-group functionalized with different moieties to modulate charge or to introduce specific molecular moieties like ligands.

Summary

In one aspect, the disclosure relates to a composition comprising a plurality of RNA particles, wherein each particle comprises:

(i) RNA;

and

(ii) one or more lipids (a) complexed with RNA by non-covalent interactions or (b) enclosing or encapsulating RNA, thereby forming RNA particles,

wherein polysarcosine is conjugated to at least one of the one or more lipids, and wherein the at least one lipid is a fatty acid, a glycerolipid, a glycerophospholipid, a sphingolipid, a saccharolipid, a polyketide, a sterol lipid or a prenol lipid.

In one example, the disclosure relates to a composition comprising a plurality of RNA particles, wherein each particle comprises:

(i) RNA;

and

(ii) one or more components which associate with RNA to form RNA particles,

wherein polysarcosine is conjugated to at least one of the one or more components.

In one example, the RNA particles are non-viral RNA particles. In one example, the one or more components which associate with RNA to form particles comprise one or more polymers. In one example, the one or more polymers comprise a cationic polymer. In one example, the cationic polymer is an amine-containing polymer. In one example, the one or more polymers comprise one or more polymers selected from the group consisting of poly-L-lysine, polyamidoamine, polyethyleneimine, chitosan and poly(β-amino esters).

In one example, the one or more components which associate with RNA to form particles comprise one or more lipids or lipid-like materials. In one example, the one or more lipids or lipid-like materials comprise a cationic or cationically ionizable lipid or lipid-like material. In one example, the cationically ionizable lipid or lipid-like material is positively charged only at

acidic pH and does not remain cationic at physiological pH. In one example, the one or more lipids or lipid-like materials comprise one or more additional lipids or lipid-like materials. In one example, the polysarcosine is conjugated to at least one of the one or more additional lipids or lipid-like materials.

In a further aspect, the disclosure relates to a composition comprising a plurality of RNA-lipid particles, wherein each particle comprises:
(a) RNA;
(b) a cationic or cationically ionizable lipid;
and
(c) a polysarcosine-lipid conjugate, wherein the lipid is a fatty acid, a glycerolipid, a glycerophospholipid, a sphingolipid, a saccharolipid, a polyketide, a sterol lipid or a prenol lipid, optionally wherein each particle further comprises: (d) a non-cationic lipid.

In a further example, the disclosure relates to a composition comprising a plurality of RNA-lipid particles, wherein each particle comprises:
(a) RNA;
(b) a cationic or cationically ionizable lipid or lipid-like material;
and
(c) a polysarcosine-lipid conjugate or a conjugate of polysarcosine and a lipid-like material.

In another aspect, the present disclosure refers to a composition as described herein for use in therapy for delivering therapeutic or prophylactic RNA to cells of a subject. In yet another aspect, the present disclosure refers to a composition as described herein for use in therapy for delivering a therapeutic peptide or protein to a subject, wherein the RNA encodes the therapeutic peptide or protein.

In another aspect, the present disclosure refers to use of a composition as described herein in the manufacture of a medicament for treating or preventing a disease or disorder in a subject, wherein the RNA is therapeutic or prophylactic RNA, and wherein delivering the RNA to cells of the subject is beneficial in treating or preventing the disease or disorder. In yet another aspect, the present disclosure refers to use of a composition as described herein in the manufacture of a medicament for treating or preventing a disease or disorder in a subject, wherein the RNA encodes a therapeutic or prophylactic peptide or protein and wherein

delivering the therapeutic peptide or protein to the subject is beneficial in treating or preventing the disease or disorder.

In one example, each particle further comprises:

(d) a non-cationic lipid or lipid-like material.

In one example, the cationic or cationically ionizable lipid or lipid-like material comprises from about 20 mol % to about 80 mol % of the total lipid and lipid-like material present in the particles.

Claims

1. A composition comprising a plurality of RNA particles, wherein each particle comprises:
(i) RNA;
and
(ii) one or more lipids (a) complexed with RNA by non-covalent interactions or (b) enclosing or encapsulating RNA, thereby forming RNA particles,
wherein polysarcosine is conjugated to at least one of the one or more lipids, and wherein the at least one lipid is a fatty acid, a glycerolipid, a glycerophospholipid, a sphingolipid, a saccharolipid, a polyketide, a sterol lipid or a prenol lipid.

2. The composition of claim 1, wherein the RNA particles are non-viral RNA particles.

3. The composition of claim 1 or 2, wherein the one or more lipids comprise a cationic or cationically ionizable lipid, optionally wherein the cationically ionizable lipidis positively charged only at acidic pH and does not remain cationic at physiological pH and/or, wherein the one or more lipids comprise one or more additional lipids, optionally wherein the polysarcosine is conjugated to at least one of the one or more additional lipids.

4. A composition comprising a plurality of RNA-lipid particles, wherein each particle comprises:
(a) RNA;
(b) a cationic or cationically ionizable lipid;
and
(c) a polysarcosine-lipid conjugate, wherein the lipid is a fatty acid, a glycerolipid, a glycerophospholipid, a sphingolipid, a saccharolipid, a polyketide, a sterol lipid or a prenol lipid,
optionally wherein each particle further comprises: (d) a non-cationic lipid.

5. The composition of any one of claims 1 to 4, wherein the particles do not comprise a polyethyleneglycol-lipid conjugate or a conjugate of polyethyleneglycol, and preferably do not comprise polyethyleneglycol.

6. The composition of claim 4 or 5, wherein
a) the cationic or cationically ionizable lipid comprises from 20 mol % to 80 mol % of the total lipid present in the particles;
b) the non-cationic lipid comprises from 0 mol % to 80 mol % of the total lipid present in the particles; and/or

c) the polysarcosine-lipid conjugate or comprises from 0.25 mol % to 50 mol % of the total lipid present in the particles.

7. The composition of any one of claims 1 to 6, wherein the RNA is mRNA.

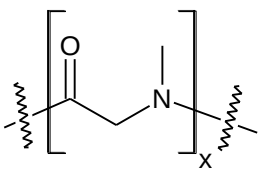
8. The composition of any one of claims 1 to 7, wherein the one or more lipids or the non-cationic lipid comprises
a) a phospholipid;
b) cholesterol or a cholesterol derivative; and/or
c) a mixture of a phospholipid and cholesterol or a cholesterol derivative, or
wherein the non-cationic lipid comprises a mixture of DSPC and cholesterol.

9. The composition of any one of claims 3 to 6, wherein the cationic or cationically ionizable lipid comprises N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), or a mixture thereof.

10. The composition of claim 9, wherein the phospholipid is selected from the group consisting of distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DPPC), or a mixture thereof.

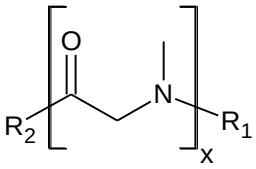
11. The composition of any one of claims 1 to 10, wherein the polysarcosine comprises between 2 and 200 sarcosine units.

12. The composition of any one of claims 4 to 11, wherein the polysarcosine-lipid conjugate comprises
a) the general formula (I):



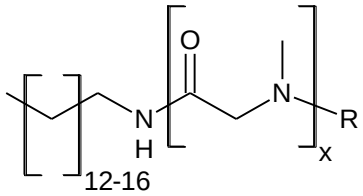
wherein x refers to the number of sarcosine units;

b) the general formula (II):



wherein one of R_1 and R_2 comprises a hydrophobic group and the other is H, a hydrophilic group or a functional group optionally comprising a targeting moiety and x refers to the number of sarcosine units; and/or

c) the general formula (III):



wherein R is H, a hydrophilic group or a functional group optionally comprising a targeting moiety and x refers to the number of sarcosine units.

13. The composition of any one of claims 1 to 12, wherein the polysarcosine-lipid conjugate is a member selected from the group consisting of a polysarcosine-diacylglycerol conjugate, a polysarcosine-dialkyloxypropyl conjugate, a polysarcosine-phospholipid conjugate, a polysarcosine-ceramide conjugate, and a mixture thereof.

14. The composition of any one of claims 1 to 13, wherein the particles are nanoparticles, optionally having a size of from 30 nm to 500 nm.

15. The composition of any one of claims 1 to 14, wherein the particles comprise a nanostructured core.

16. A composition of any one of claims 1 to 15 for use in therapy for delivering therapeutic or prophylactic RNA to cells of a subject.

17. A composition of any one of claims 1 to 15 for use in therapy for delivering a therapeutic peptide or protein to a subject, wherein the RNA encodes the therapeutic peptide or protein.

18. The composition for use according to claim 16 or 17, wherein the subject is a mammal.

19. The composition for use of claim 18, wherein the mammal is a human.

20. Use of a composition of any one of claims 1 to 15 in the manufacture of a medicament for treating or preventing a disease or disorder in a subject, wherein the RNA is therapeutic or prophylactic RNA, and wherein delivering the RNA to cells of the subject is beneficial in treating or preventing the disease or disorder.

21. Use of a composition of any one of claims 1 to 15 in the manufacture of a medicament for treating or preventing a disease or disorder in a subject, wherein the RNA encodes a therapeutic or prophylactic peptide or protein and wherein delivering the therapeutic peptide or protein to the subject is beneficial in treating or preventing the disease or disorder.

22. The use of claim 20 or 21, wherein the subject is a mammal.

23. The use of claim 22, wherein the mammal is a human.