[0004] To be useful commercially, reproducible and scalable methods for the assembly of uniform and stable polyplex complexes with tight control of physical parameters are needed. In some embodiments, the present disclosure provides improved methods for preparing polyplexes, including methods that are scalable for commercial scale production.

SUMMARY

[0004a] In an aspect of the present disclosure, there is provided a method for making one or more polyplexes, the method comprising:

(a) providing:

i. a highly branched poly amino ester (HPAE) polymer in a first liquid stream;

ii. a nucleic acid component in a second liquid stream;

(b) contacting the HPAE polymer in the first liquid stream with the nucleic acid component in the second liquid stream to form a polyplex; and

(c) isolating the polyplex to provide a stabilized polyplex,

wherein the particle size of the stabilized polyplex is less than 2 μm, and the zeta potential of the stabilized polyplex is from 0 mV to 100 mV.

[0005] In some examples, the present disclosure provides a method for making one or more polyplexes, the method comprising:

(a) providing:

i. a polymer in a first liquid stream;

ii. a nucleic acid component in a second liquid stream;

(b) contacting the polymer in the first liquid stream with the nucleic acid component in the second liquid stream to form a polyplex having a size and charge that is suitable for therapeutic administration; and

(c) isolating the polyplex to provide a stabilized polyplex.

[0006] In some examples, the method further comprises assessing and harvesting one or more polyplexes. In some examples, the method of the present disclosure is a continuous process whereby each polyplex formed is isolated from

other polyplexes formed in the method. In some examples, each polyplex is separated from other polyplexes during a stabilization phase after contacting the first and second liquid streams.

[0007] In some examples, the method further comprises filtering, washing, freezing and/or lyophilizing the stabilized polyplexes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] **FIG. 1** describes an exemplary method of the present disclosure for manufacturing polyplexes.

CLAIMS:

What is claimed:

1. A method for making one or more polyplexes, the method comprising:
(a) providing:
i. a highly branched poly amino ester (HPAE) polymer in a first liquid stream;
ii. a nucleic acid component in a second liquid stream;
(b) contacting the HPAE polymer in the first liquid stream with the nucleic acid component in the second liquid stream to form a polyplex; and
(c) isolating the polyplex to provide a stabilized polyplex,
wherein the particle size of the stabilized polyplex is less than 2 μm, and the zeta potential of the stabilized polyplex is from 0 mV to 100 mV.

2. The method of claim 1, further comprising assessing and harvesting one or more polyplexes.

3. The method of claim 1 or 2, wherein the first liquid stream and second liquid streams are solutions.

4. The method of any one of claims 1-3, further comprising flowing the first liquid stream and the second liquid stream through a flow-regulating device at a rate that provides a polyplex having a size and charge for transdermal administration.

5. The method of claim 4, wherein the flow-regulating device is selected from the group consisting of a positive displacement pump, a syringe driven pump, a pressure driven pump, and a gravity feed pump.

6. The method of any one of claims 1-5, wherein the contacting step uses a nozzle, a micro fluidics mixing device, a touch tube, a liquid bridge, a vertical mixer, a rotating double tube, or an atomizer.

7. The method of any one of claims 1-6, wherein the stabilized polyplex is slightly positively charged.

8. The method of any one of claims 1-7, further comprising filtering, washing, freezing and/or lyophilizing the stabilized polyplexes.

9. The method of any one of claims 1-8, wherein the polymer is positively charged and the nucleic acid component is negatively charged.

10. The method of any one of claims 1-9, wherein the polymer and nucleic acid component are mixed in a ratio of from about 0.1:1 to about 200:1 (w/w).

11. The method of claim 10, wherein the polymer and the nucleic acid component are mixed in a ratio of from about 20:1 to about 80:1 (w/w).

12. The method of claim 10, wherein the polymer and the nucleic acid component are mixed in a ratio of about 30:1 (w/w).

13. The method of any one of claims 1-12, wherein the polymer and nucleic acid component are present at a ratio of from about 0.1:1 to about 200:1 (w/w) in the polyplex.

14. The method of claim 13, wherein polymer and nucleic acid component are present at a ratio of about 30:1 (w/w) in the polyplex.

15. The method of any one of claims 1-14, wherein the particle size of the stabilized polyplex is about 120 nm to about 260 nm.

16. The method of any one of claims 1-15, wherein the zeta potential of the stabilized polyplex is from about 30 mV to about 45 mV.

17. The method of any one of claims 1-16, wherein the stabilized polyplex is spherical.

18. The method of any one of claims 1-17, wherein the nucleic acid component is a plasmid, nanoplasmid, nucleic acid, minicircle, or gene editing system.

19. The method of claim 18, wherein the nucleic acid component comprises a gene associated with a genetic disease or disorder.

20. The method of claim 19, wherein the genetic disease or disorder is caused by a mutation in one or more genes that results in low, absent, or dysfunctional protein expression.

21. The method of claim 20, wherein the gene is selected from the group consisting of COL7A1, LAMB3, ADA, SERPINA1, CFTR, HTT, NF1, PHA, HBS, FERMT1, KRT14, DSP, SPINK5, and FLG.

22. The method of claim 21, wherein the gene is COL7A1 and the genetic disease or disorder is a form of epidermolysis bullosa.

23. The method of claim 22, wherein the sequence of the gene is optimized for maximum protein expression upon delivery of the polyplex to a cell.

24. The method of any one of claims 1-23, wherein the polymer has an alpha parameter defined from the Mark-Houwink equation of less than about 0.5.

25. The method of any one of claims 1-24, wherein the polymer has an alpha parameter defined from the Mark-Houwink equation from about 0.2 to about 0.5.

26. The method of any one of claims 1-25, wherein the polymer has a PDI of about 3.0 to about 5.8.

27. The method of any one of claims 1-26, wherein the polymer has a M_W of between about 5 kDa and 50 kDa.

28. The method of any one of claims 1-27, wherein the polymer has a M_W of between about 19kDa and about 25 kDa.

29. The method of claim 1, wherein the concentration of the HPAE polymer in the first liquid stream is about 1 μg/μL to about 15 μg/μL; and/or

wherein the concentration of the nucleic acid component in the second liquid stream is about 0.1 mg/mL to about 0.5 mg/mL.

30. The method of claim 1, wherein the flow rate of the first liquid stream and second liquid stream is about 60 μL/min to about 1000 μL/min.

31. The method of claim 30, wherein the flow rate is about 400 μL/min to about 700 μL/min.