Strategies for producing clinical and commercial RNA-LNP drug products

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Introduction

- The promise of messenger RNA (mRNA) lipid nanoparticle (LNP) therapies include prophylactic, rare disease, and oncology applications.
- However, encapsulation of mRNA drug substances by lipids is among the most difficult unit operation to bring to commercial-scales.
- In this work, we aim to demonstrate that the NanoAssemblr® commercial formulation system and NxGen™ commercial cartridge 48 L/h simplify this unit operation.

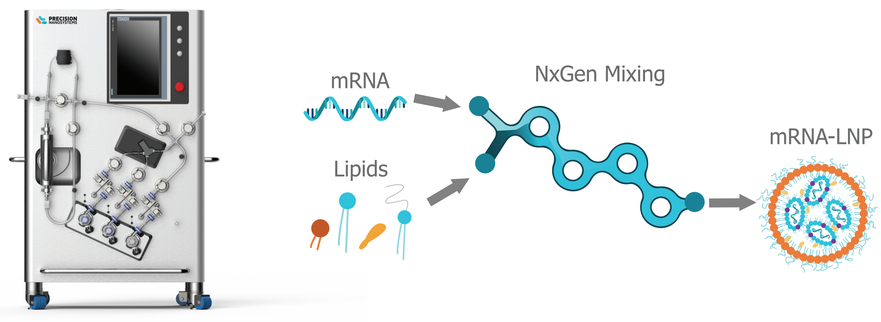

Figure 1. NanoAssemblr commercial formulation system (left) and NxGen microfluidic mixing system (right)

Objectives

- Demonstrate consistent mixing → The quality of mixing in the NxGen series of mixers was probed by formulating POPC:Chol liposomes at a range of flow rates
- Show scalability from pre-clinical to clincal/commercial → Increasingly large batches of plasmid DNA-LNPs were prepared with NxGen mixers
- Prepare consistent RNA-LNP drug products at all scales → Flow rates for saRNA-LNP production were stepwise increased by an order of magnitude

Methods and Results

Nanoparticle synthesis and purification:
POPC(1-palmitoyl-2-oleoyl-glycero-3-phosphocholine):Chol liposomes were prepared at a range of flow rates on NxGen mixers. Green fluorescent protein (GFP) plasmid DNA (pDNA) LNPs or self-amplifying mRNA (saRNA)-LNPs were prepared using NanoAssemblr® instruments and NxGen™ mixers. Specific formulation conditions are noted in the tables right and below.

RNA-LNP characterization and *in vitro* activity: RNA-LNP size and polydisersity index (PDI) were determined using DLS (Malvern Zetasizer Ultra). The encapsulation efficiency (EE%) of the RNA was determined using Ribogreen™ reagent.

***In vitro* and *in vivo* expression and immunogenicity:** *In vitro* potency was assessed with a kinase deficient baby hamster kidney cell (BHK 570) cell model. To determine the immunogenicity of the saRNA-LNPs, female BALB/c mice (n=5) were immunized by IM injection on day 0 with LNPs encapsulating 1µg nCoV saRNA and boosted at day 28. IgG levels in serum on day 21 and day 42 were measured by ELISA.

Condition	NanoAssemblr® system	NxGen mixer cartridge	Total flow rate [L/h]	Batch volume [mL]	RNA Encapsulated [mg]
1	Ignite+	NxGen	0.72	30	1.1
2	Ignite+	NxGen 500	6.9	30	1.1
3	Ignite+	NxGen 500	12	30	1.1
4	Blaze	NxGen 500	6.9	30	1.1
5	Commercial formulation system	NxGen commercial cartridge 12 L/h [Nxgen 500]	12	100	3.3
6	Commercial formulation system	NxGen commercial cartridge 48 L/h	48	100	3.3
7	Modular commercial formulation skid	NxGen commercial cartridge 48 L/h	48	150	5.0

Table 1. saRNA-LNP formulation conditions

1. NxGen Mixing Architecture Ensures Consistent Particles Across a Wide Range of Flow Rates

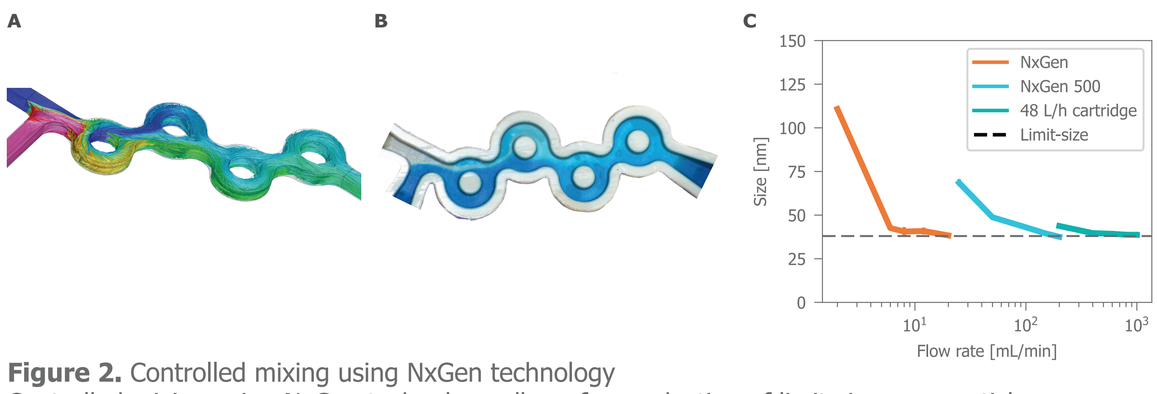

Figure 2. Controlled mixing using NxGen technology
Controlled mixing using NxGen technology allows for production of limit-size nanoparticles across a wide range of flow rates. **A)** computational fluid dynamic modeling with water and ethanol. **B)** Dye studies using the NxGen commercial cartridge 48 L/h. **C)** POPC:Chol liposome formation. The size of POPC:Chol liposomes prepared using the NxGen, NxGen 500, and NxGen commercial cartridge 48 L/h at a range of flow rates

2. Consistent LNP Formulation Conditions for >6g IVT Process

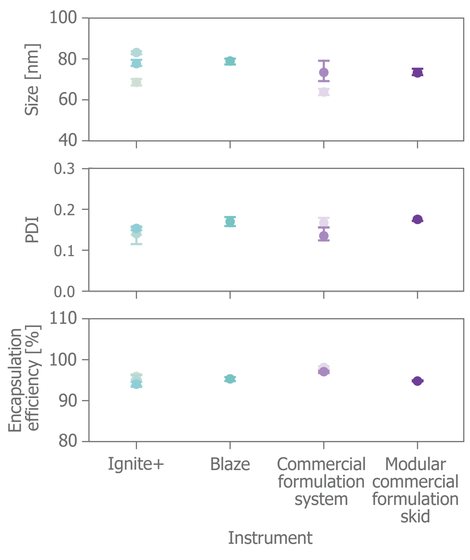

Figure 3. pDNA-LNPs prepared with the NxGen commercial cartridge 48 L/h in batches of up to 50 L in volume
Size, polydispersity index and encapsulation efficiency of 3 pDNA-LNP formulations prepared using the NxGen commercial cartridge 48 L/h as a function of batch size (volume). Values are n = 1 for 5, 10 and 50 L batches while the 2 mL NxGen control sample is n =3.

Key Formulation Parameters for pDNA-LNPs	
Lipid Composition	Precision NanoSystems Custom Composition Ionizable lipid : Helper 1 : Helper 2 : Stabilizer
Initial Lipid Concentration	2x nominal concentration
Genetic Cargo	6.1 kb eGFP pDNA
N/P	8
Organic Solvent	Ethanol
Aqueous Phase	2x nominal pDNA concentration in acidic buffer
Flow Rate Ratio	3:1 aqueous to organic

3. Critical Quality Attributes of saRNA-LNPs Are Consistent Across NanoAssemblr Systems

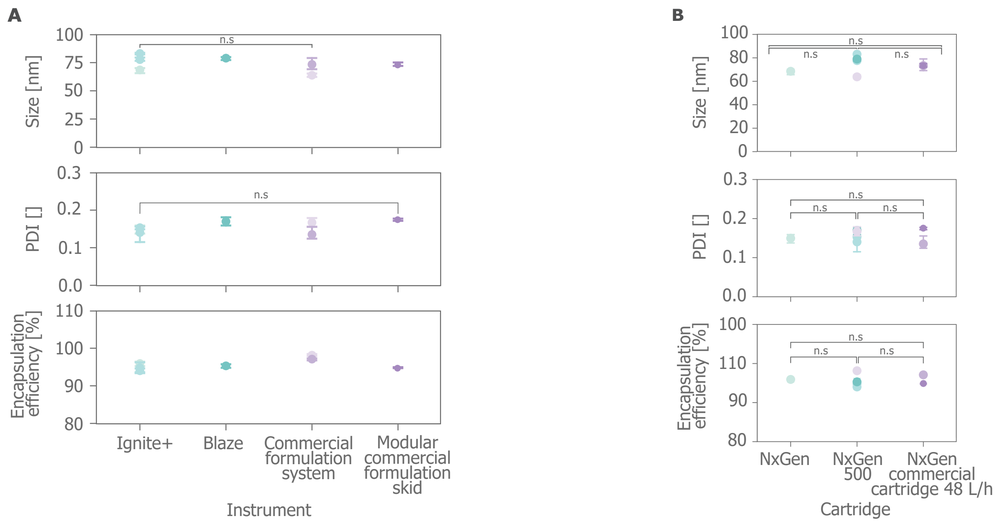

Figure 4. Physicochemical characterization of saRNA-LNPs prepared using NxGen Technology
A) Size, PDI, and encapsulation efficiency as a function of instrument system used to prepare the saRNA-LNP. **B)** Size, PDI and encapsulation efficiency as a function of NxGen mixer cartridge.

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Genetic Cargo	6.1 kb eGFP pDNA
N/P	8
Organic Solvent	Ethanol
Aqueous Phase	2x nominal pDNA concentration in acidic buffer
Flow Rate Ratio	3:1 aqueous to organic
TFF Concentration and Diafiltration	Cytiva Delta cassette 30 kDa, 93 cm^2
Cryopreservation Buffer	Precision NanoSystems custom
Sterile Filtration	Cytiva Acrodisc 0.22 µm

4. Commercial Scale saRNA-LNPs Are Biologically Potent *In Vitro* and *In Vivo*

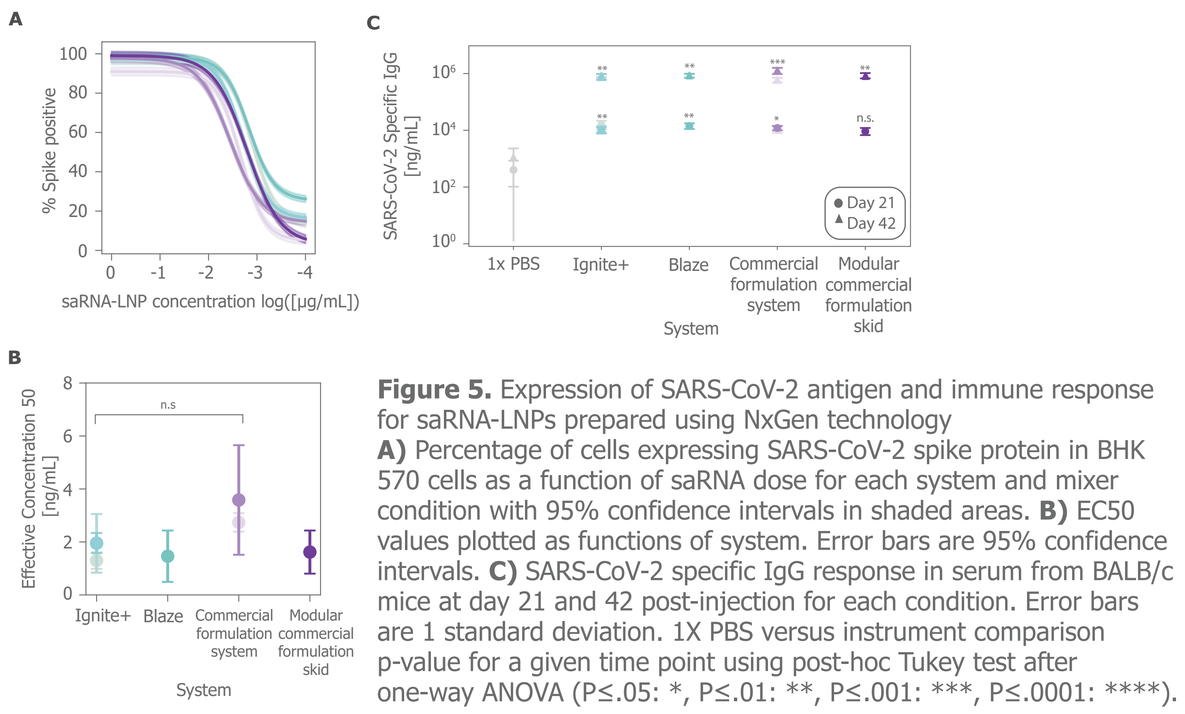

Figure 5. Expression of SARS-CoV-2 antigen and immune response for saRNA-LNPs prepared using NxGen technology
A) Percentage of cells expressing SARS-CoV-2 spike protein in BHK 570 cells as a function of saRNA dose for each system and mixer condition with 95% confidence intervals in shaded areas. **B)** EC50 values plotted as functions of system. Error bars are 95% confidence intervals. **C)** SARS-CoV-2 specific IgG response in serum from BALB/c mice at day 21 and 42 post-injection for each condition. Error bars are 1 standard deviation. 1X PBS versus instrument comparison p-value for a given time point using post-hoc Tukey test after one-way ANOVA (P≤.05: *, P≤.01: **, P≤.001: ***, P≤.0001: ****).

Conclusion

- Critical quality attributes of the saRNA-LNPs were maintained across all scales and flow rates for all analytical readouts.
- The NxGen commercial cartridge 48 L/h and NanoAssemblr commercial formulation system provide a scalable solution for production of RNA-LNP drug products under cGMP conditions.

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