A Scalable Microfluidics Platform for the Development of Nanoparticles

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Introduction

Purpose

Nucleic acid therapies permit access to previously undruggable pathways and allow manipulation of cellular machinery to produce target proteins including antigens for vaccines. To overcome challenges of gene delivery, scientists are optimizing nanoparticles for delivery by fine-tuning their size, composition and surface properties. Although these efforts have yielded substantial results in the laboratory to date, a significant need exists for robust manufacturing technologies to transit these discoveries from lab to clinic. In this context, we present a microfluidic based NanoAssemblr™ platform for production of mL to L of nanoparticles that retains consistent quality, efficacy and safety profiles, at scale, throughout the development process.

Factor VII siRNA lipid nanoparticles (LNPs) were prepared on the NanoAssemblr™ Benchtop (Precision NanoSystems, Inc., Vancouver, Canada) and formulation parameters such as concentrations, flow rate ratio and total flow rate were optimized. These optimized process parameters were transferred onto the NanoAssemblr™ Blaze™ and 8X Scale-up System to scale this formulation to 100 mL and 1000 mL respectively. Physico-chemical properties and *in vivo* activity were measured for particles produced by each instrument to test consistency across the platform. Particle composition was determined by HPLC. Particle size and polydispersity was analyzed using dynamic light scattering and RNA encapsulation efficiency was determined from standard RiboGreen based assay. Finally, *in vivo* activity was tested by administering LNPs in wild-type mice by i.v. injection and measuring serum Factor VII levels.

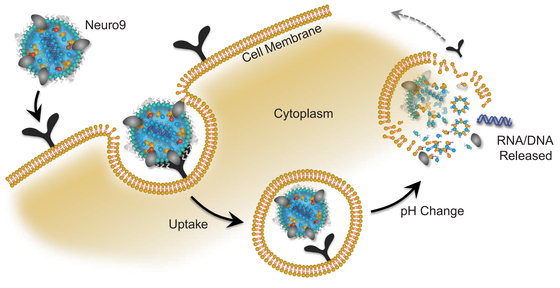
Results

We obtained LNP particles of about 60 nm (PDI <0.1) with encapsulation efficiency >95% on the NanoAssemblrTM Benchtop. No differences were observed in physicochemical properties of these particles when scaled-up by 10x on Blaze (100 mL) or by 100x on 8X Scale-up System (1000 mL). The particles exhibited consistent lipid composition and N/P ratio within the target specifications. In addition, the particles manufactured across the microfluidic platform showed a similar dose-dependent gene knockdown, achieving > 90% reduction in protein levels at a dose of 1 mg/kg.

These studies demonstrated that the NanoAssemblrTM platform provides seamless scale-up and can produce large-scale volumes of lipid nanoparticles with consistent results. The 8X scale-up system can prepare up to 25 L of product under 4.5 hours at 96 mL/min and incorporates a disposable fluid path that eliminates the need for costly and time consuming cleaning validation.

Gene Delivery via Lipid Nanoparticles

Lipid Nanoparticles (LNPs) are a platform that can be used to deliver nucleic acids to cells. LNPs mimic low density lipoproteins (LDLs), which are taken up by an endogenous pathway. LNPs are pH sensitive, and designed to release their payload into the cytoplasm.



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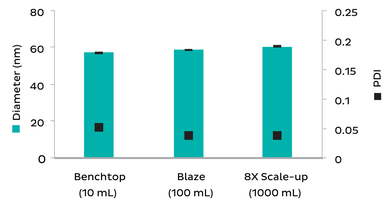
Seamless scale-up of siRNA LNPs

Factor VII siRNA LNPs maintain properties when scaled up by 10x and 100x

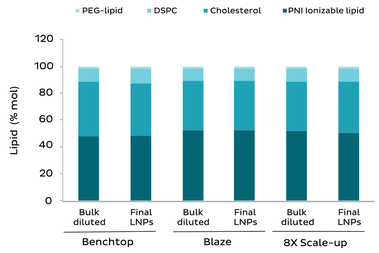
Lipid Nanoparticles (LNPs) containing siRNA against Factor VII optimized on the NanoAssemblr™ Benchtop, were also made on the Blaze, and 8x Scale-Up systems using the same parameters.

Size and PDI maintained across NanoAssemblr systems and scales



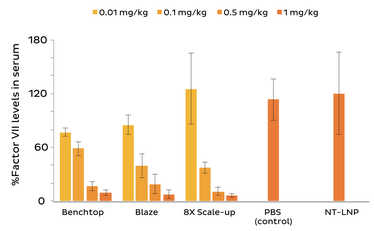
Hydrodynamic diameter and polydispersity index (PDI) of siRNA LNPs were consitent between NanoAssemblr Benchtop, Blaze or 8x Scale-up systems at 10, 100, and 1000 mL batches respectively. siRNA encapsulation efficiency was measured at >95%.

Composition maintained across NanoAssemblr systems and scales



siRNA LNPs maintained composition between the NanoAssemblr Benchtop, Blaze and 8x Scale-up systems at 10, 100, and 1000 mL batches respectively. Composition was also maintained following solvent removal. Lipid compositions were within ± 10% of target specifications. LNPs were disrupted and lipids were separated and analyzed on an HPLC column using ELS detector.

***In vivo* Knockdown efficacy maintained across NanoAssemblr systems and scales**



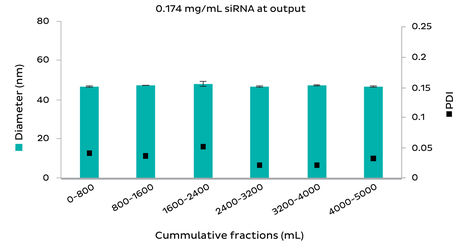
LNPs containing siRNA against FVII or non-targeting (NT) control were administered to CD1 mice by tail vein injection at the doses indicated. Knockdown efficacy was maintained between LNPs produced on the NanoAssemblr Benchtop, Blaze and 8x Scale-up at 10, 100, and 1000 mL batches respectively.

High concentration siRNA LNP manufacturing

Factor VII siRNA LNPs manufactured at high concentrations on the Scale-Up system retain their physico-chemical properties

Lipid Nanoparticles (LNPs) containing siRNA against Factor VII optimized on the NanoAssemblr™ Benchtop at 0.174 mg/mL siRNA were made on the Scale-Up system with a single chip at the same concentration and at 1.39 mg/mL.

Size and PDI maintained at both concentrations



Hydrodynamic diameter and polydispersity index (PDI) of siRNA LNPs were consitent between fractions collected from continuous flow manufacturing on the 8x Scale-Up system. Diameter and PDI were measured by dynamic light scattering.

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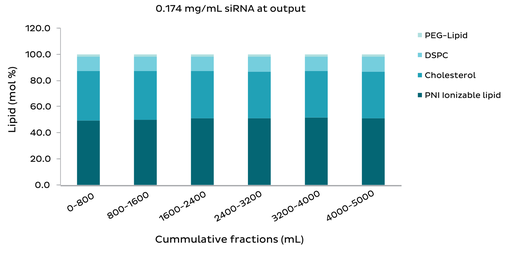
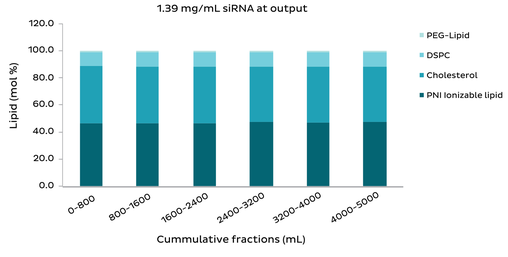
Lipid composition maintained at both concentrations



Composition of siRNA LNPs were consitent between fractions collected from 5L continuous flow manufacturing run on the 8x Scale-Up system. LNPs were disrupted and lipids were separated and analyzed on an HPLC column using ELS detector.



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Methods

The Microfludic Platform

The NanoAssemblr™ platform uses microfluidics to precisely control the manufacturing of nanoparticles from microlitre to litre scales.

SCAN WITH layar Enriched content: NanoAssemblr microfluidic particle production animation.

NanoAssemblr™ Systems

Factor VII siRNA formulation

CL/DSPC/CHOL/PEG-lipid solutions were prepared as described previously. Factor VII siRNA (Axolabs) was dissolved in acetate buffer (pH 4) at 0.232 mg/mL, 0.928 mg/mL and 1.856 mg/mL. The siRNA concentration was determined using UV spectroscopy (Nanodrop Lite, Thermo Fisher Scientific Inc).

For scale-up study, about 10 mL of formulation was prepared on NanoAssemblr™ Benchtop at aqueous:organic FRR of 3:1 and TFR of 12 mL/min. The formulation was scaled-up to 100 mL on Blaze and 1000 mL on 8X Scale-up System using the Benchtop microfluidic parameters. LNPs were manufactured at siRNA concentration of 0.7 mg/mL in all three instruments and diluted with PBS before buffer-exchange on tangential flow filtration system (KrosFlo® Research III Tangential Flow Filtration (TFF) System, SpectrumLabs). Finally, the particles were concentrated to 1 mg/mL, sterile filtered and stored at 4 °C before further analysis.

For single mixer experiments, a total of 5000 L of LNPs were prepared on a single scale-up chip at 0.17 mg/mL and 1.39 mg/mL siRNA concentration. Particle dilution and ethanol removal was done on TFF, as described above.

Particle characterization

A sample of bulk diluted and final product was used to determine physiochemical characteristics (size, PDI, encapsulation), lipid concentration and siRNA concentration. Particle size was determined using dynamic light scattering technique (Malvern Zetasizer Nano ZS) and encapsulation using RiboGreen assay. Lipids were separated and analyzed on an HPLC column using an ELS detector. For siRNA quantification, LNPs were disrupted using a surfactant and analyzed in HPLC using photodiode (UV) array detector.

Factor VII gene knockdown

CD1 mice (18-23 g; 6-8weeks) were divided into 14 groups (n=3) and siRNA LNPs were administered intravenously using a single bolus injection via tail vein at a dose of 0.01mg/kg, 0.1 mg/kg, 0.5 mg/Kg and 1 mg/Kg. Non-targeting (NT) siRNA containing chemical modifications and number of nucleotide similar to Factor VII siRNA was used for preparation of negative control. After 24 hours, blood (~ 500 µL) was collected by cardiac puncture after euthanizing mice. The blood samples were allowed to stand for 20-40 min at RT to allow the blood to clot. Serum was separated by centrifuging at 2000xg for 10 min at 15 °C and was stored at -80 °C for analysis later. Analysis of Factor VII protein levels in the serum were performed using Biophen Factor VII assay (Aniara, OH, USA).

Conclusion

The NanoAssemblr™ platform is capable of creating highly reproducible LNPs carrying nucleic acid payloads at scales from wet bench development through to clinical trials.

Non-turbulent microfluidic mixing and precise fluid flow produce LNPs consistent in size, polydispersity, composition, and in vivo efficacy across NanoAssemblr systems.

Conditions affecting LNP assembly were optimized on the NanoAssemblr Benchtop at mL scales using instument parameters such as FRR and TFR. Instrument parameters were transfered seamlessly to the Blaze and 8x Scale-Up systems to produce larger batches with identical characteristics and results.

Using a single chip, the continuous flow Scale-Up system produced LNPs consistent in size, PDI and composition throughout the process as determined by collecting and analyzing separate fractions of the output.

LNP formulations were also produced at high concentrations while maintaining particle quality acceptable for in vivo use.

At 1.39 mg/mL output for a single chip, and a TFR of 12 mL/min, the 1x Scale-Up system produced siRNA LNPs at a rate of 16.7 mg siRNA per min (1000 mg/h). Hence, the 8x Scale-Up system can produce LNPs at a rate of 133 mg siRNA per min (8000 mg/h).

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