Rapid Development And Seamless Scale-up Of Genetic Nanomedicines

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Purpose and Objectives

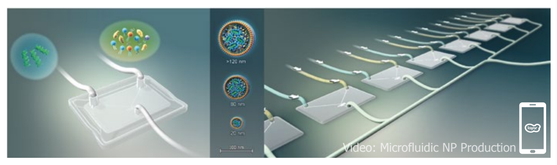
- Nucleic acid therapies permit access to previously undruggable pathways but successful delivery of those therapies remains challenging
- Lipid nanoparticles (LNPs) can overcome challenges in delivery of nucleic acid therapeutics
- However, technologies enabling rapid development of LNP formulations, and streamlined avenue to scale manufacturing to meet demands are still required
- Previous work demonstrated the optimization of siRNA-LNP formulations at 2-10 mL scale on the NanoAssemblr™ Benchtop with further scaling to 100 mL on the Blaze™ and 1000 mL on the 8X Scale-up System
- Here, we demonstrate the stepwise scaling of an optimized luciferase (Luc) mRNA LNP formulation manufactured at 7 mL on the NanoAssemblr Benchtop and scaled it up 10X on NanoAssemblr Blaze and 70X on 8X Scale-up system

Methods

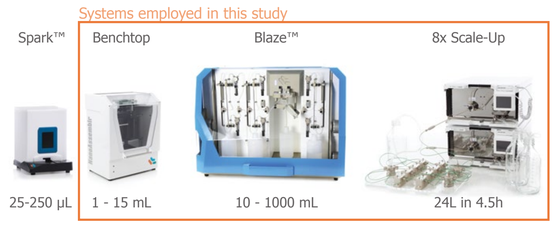
For Detailed Materials and Methods Scan with Layar App

The Microfludic Platform

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



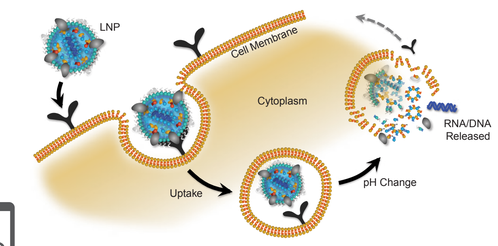
NanoAssemblr™ Systems



- mRNA encoding luciferase was encapsulated in LNPs containing ionizable cationic lipid, helper lipids, cholesterol and PEG-Lipid that assemble into low-density lipoprotein-like particles that are taken up by receptor mediated endocytosis.
- LNPs were optimized on the NanoAssemblr Benchtop, then made on the Blaze and 8x Scale-Up System with the same parameters
- mRNA LNPs across all scales were characterized by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), UPLC, and Cryo-TEM to determine physical characteristics, chemical composition, and morphology.

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.



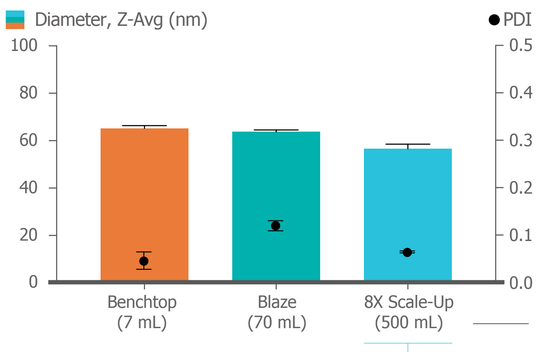
Video: LNP gene delivery and transfection

Size Analysis of mRNA-LNPs

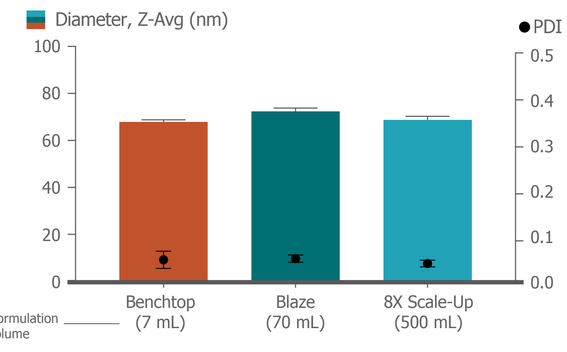
Dynamic Light Scattering (DLS)

Mean hydrodynamic diameter measured 69.7 ± 2.4nm regardless of scale or NanoAssemblr system utilized

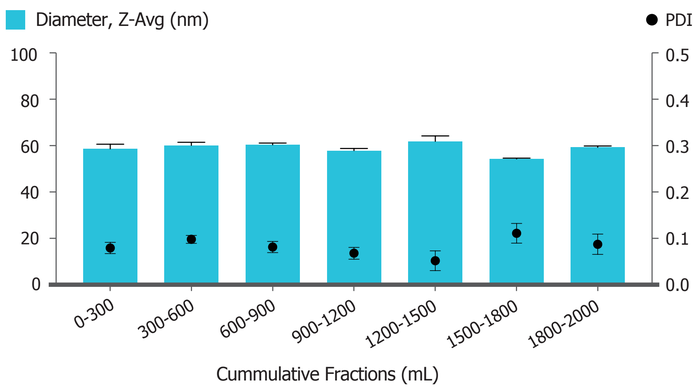
A After microfluidic mixing



B Final processed LNPs



C Size and PDI were consistent between fractions collected from 8x Scale-Up



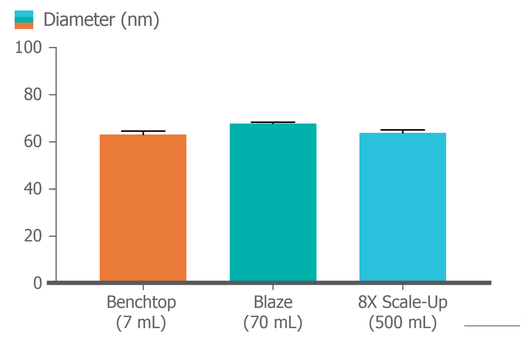
Further Details

DLS measurements of mRNA LNPs: A) immediately after collection of the entire diluted formulation manufactured on the Benchtop, Blaze and 8X Scale-up systems, and B) after tangential flow filtration (TFF) and final processing of all formulations. C) Hydrodynamic diameters and PDI were consistent between fractions collected throughout the entire continuous flow manufacturing on the 8X Scale-up system (500 mL diluted in-line to 2000 mL; prior to TFF). Error bars represent standard deviation of the mean of duplicate measurements

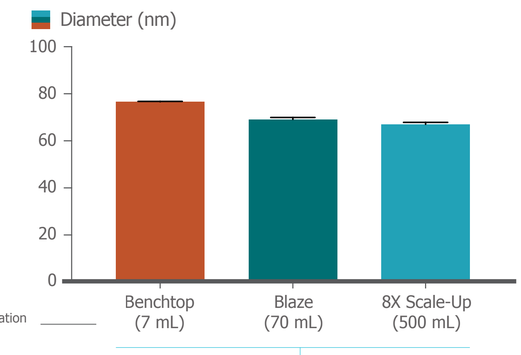
Nanoparticle Tracking Analysis (NTA)

Mean diameter measured 70.9 ± 5.0 nm with narrow size distribution across all scales

A After microfluidic mixing



B Final processed LNPs

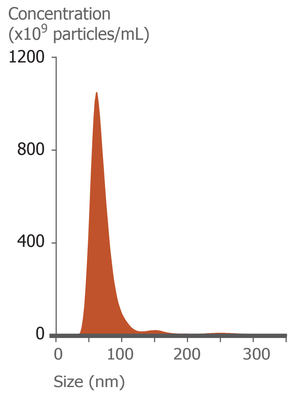


Further Details

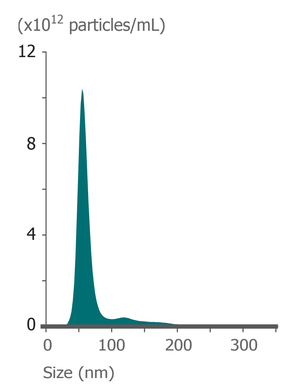
NTA measurements of mRNA-LNPs: A) Immediately after collection of the entire diluted formulation manufactured on the Benchtop, Blaze and 8X Scale-up instruments, and B) after tangential flow filtration and final processing of all formulations. Size distribution from NTA measurements of final mRNA-LNPs manufactured on C) Benchtop, D) Blaze, and E) 8X Scale-up instruments. Error bars represent standard deviation of the mean of triplicate sample measurements.

NTA Size distribution histograms of final LNPs

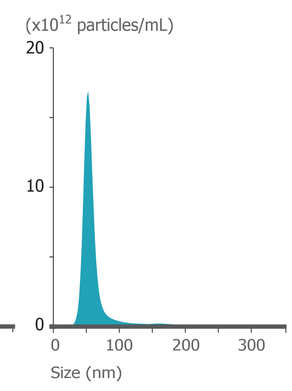
C Benchtop



D Blaze



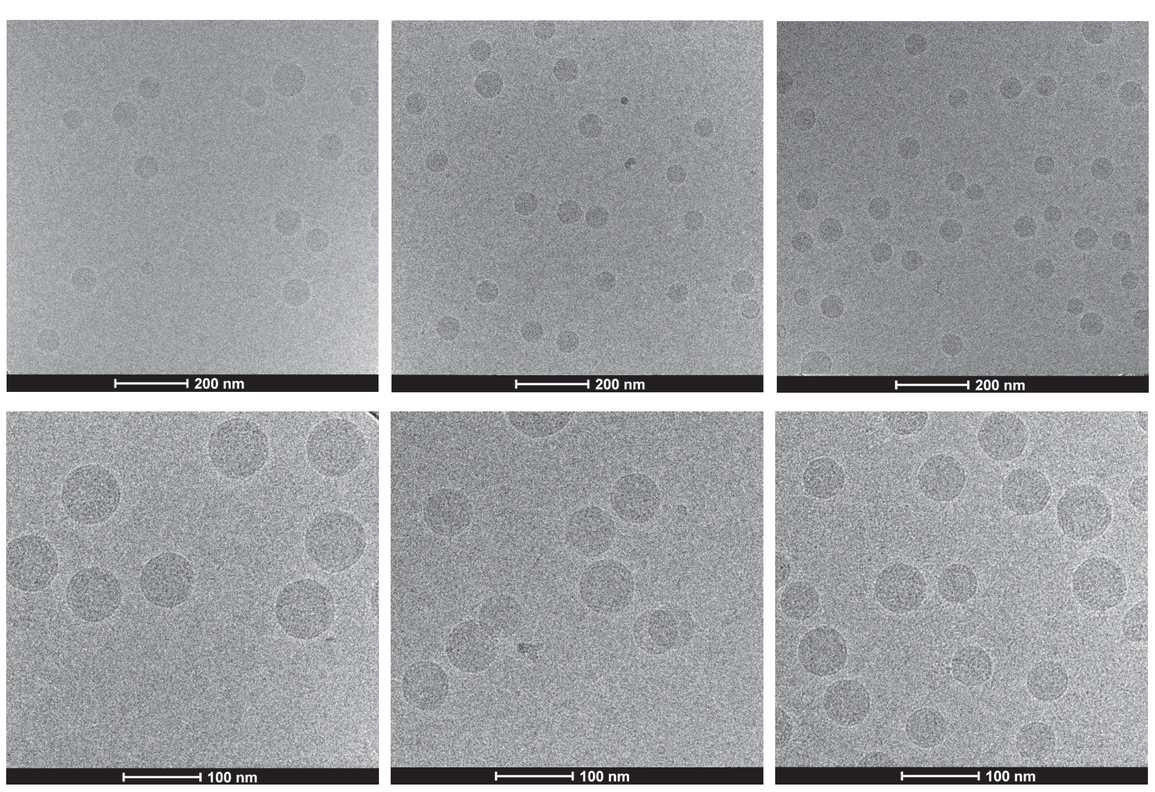
E 8x Scale-Up



High concentration mRNA LNP manufacturing

Cryo-Transmission Electron Microscopy

Electron-dense core morphology and monodisperse population maintained across manufacturing scales on the NanoAssemblr Platform



Further Details

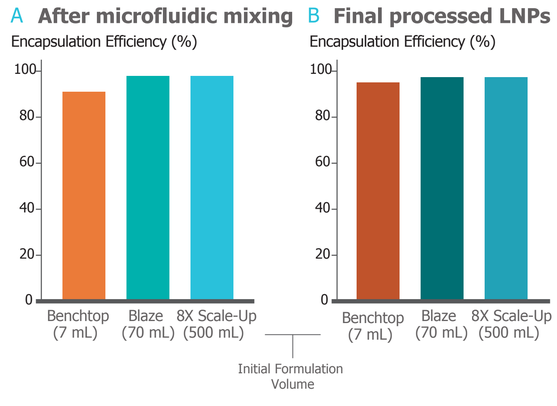
Cryo-transmission electron microscopy (cryo-TEM) micrographs provide further confidence that the conditions for mRNA-LNP manufacture were faithfully replicated at those scales. Luciferase mRNA lipid nanoparticles manufactured under continuous flow conditions using the (A) Benchtop, (B) Blaze and (C) 8x Scale-Up systems exhibit electron-dense core morphologies and a monodisperse population when scaled 10x and 70x using the NanoAssemblr platform. Scale bars indicate 200 nm (top) and 100nm (bottom).

Composition Analysis of mRNA LNPs

Encapsulation Efficiency

Exceptional encapsulation efficiency (> 90%) was maintained across manufacturing scales

A After microfluidic mixing **B Final processed LNPs**

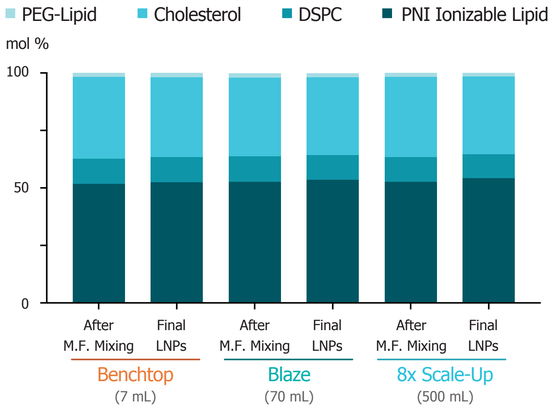


Further Details

Encapsulation efficiency, determined by Ribogreen assay, was maintained across manufacturing scales, from diluted sample collection to processed end-product.

Lipid Analysis

Lipid composition was remarkably uniform and consistent across manufacturing scales



Further Details

mRNA-LNPs maintained lipid compositions (within 10% of target) as determined by UPLC, when scaled from 7mL to 500mL on the NanoAssemblr Platform. Consistency between samples collected following continuous flow manufacturing (After MF Mixing) and downstream processing (Final LNPs) further indicate the ability to robustly scale an mRNA-LNP formulation at those volumes.

Conclusions

- Sizing data shows consistent physical properties for mRNA-LNPs manufactured across the NanoAssemblr platform as confirmed from DLS, NTA and Cryo-TEM
- Exceptional encapsulation efficiency (>90%) was achieved and maintained throughout the process from collection to final product
- Lipid composition was maintained with remarkable uniformity across the NanoAssemblr platform
- Non-turbulent microfluidic mixing and precise fluid flow result in LNPs with consistent size, polydispersity, and composition
- Conditions affecting LNP assembly were optimized on the NanoAssemblr Benchtop and transfered seamlessly to the Blaze and 8x Scale-Up systems to produce larger batches with identical characteristics and results
- Together, these results demonstrate the robustness and seamless scalability of manufacturing mRNA-LNPs using the NanoAssemblr platform

Supporting Information: Scale-Up webinar series