Robust and Scalable Manufacturing of Nucleic Acid Lipid Nanoparticles Using a Novel Microfluidic Mixing Technology

S. Abraham; H. Son; S.G. Talluri; R. Gupta; R. Young; N. Jain; A. Angell; A.W. Wild; K. Marshall; B. Versteeg; M. Kerin; M. Fellows; M. Ma; J. Ko; L. Jeffs; S. Clarke* and A. Thomas

Contact Us at:
Precision NanoSystems Inc, Vancouver, BC, Canada
info@precision-nano.com

Introduction

Purpose

- The development of gene therapies employing lipid nanoparticles (LNPs) for delivery of nucleic acids to cells is hindered by access to effective formulation reagents and scalable manufacturing methods
- Manufacturing using the Classic staggered herringbone microfluidic mixer produces homogenous LNPs with defined size, narrow distribution, and high nucleic acid loading
- NxGen™ is a new microfluidic mixer that preserves the precise and time-invariant properties of the Classic and allows for simplified scaling with 25X higher throughput (up to 20 L/h) with a single mixer
- GenVoy-ionizable lipid mix (ILM) is a RUO reagent developed for use with NxGen to produce high-quality RNA loaded LNPs

Objectives

- Demonstrate that erythropoietin (Epo)-encoded mRNA-LNPs manufactured using GenVoy-ILM™, and NxGen™ and Classic have equivalent properties
- Demonstrate the ease and robustness in scale-up manufacturing of Epo mRNA-LNP using NxGen with minimal formulation process optimization

Methods

Production: The mRNA-LNPs were prepared from the GenVoy-ILM (NWW0041, PNI) reagent and Epo-encoded mRNA (L-7209, Trilink) using NxGen and Classic mixers on the NanoAssemblr® Ignite, Blaze and GMP systems (PNI). Parameters are in Table 1.

Downstream processing: The Epo mRNA-LNP were diluted 40X times in 1X PBS and purified and concentrated using ultrafiltration (UF) with Amicon® Ultra 15 10kDa MWCO units (EMD Millipore) or tangential flow filtration (TFF) using KrosFlow® Research iII (Spectrum Labs) or Äkta™ Flux 6 (GE) TFF systems with 30kDa MWCO mPES membranes. TFF parameters are in Table 2. The final Epo mRNA-LNPs were sterile-filtered using 0.2 µm filters.

Characterization and Activity: The Epo mRNA-LNP size and polydispersity (PDI) were determined by DLS (Malvern ZetaSizer). Encapsulation efficiency was measured using a Ribogreen-based RNA assay. For in vivo activity, female C57BL mice were treated intravenously with Epo mRNA-LNPs and serum was collected 6 h post injection via saphenous vein. The total serum Epo levels were evaluated using Quantikine® IVD Epo ELISA kit (Biotechne). Blood hematocrit levels were estimated 7 days post injection using microhematocrit tubes.

Table 1. Formulation Process Parameters used in Epo mRNA-LNP manufacturing in different NanoAssemblr Systems and Mixers

NanoAssemblr® System	Benchtop	Ignite™	Blaze™	GMP
Mixers	Classic	Classic, NxGen, NxGen in-line dilution	Classic, NxGen MF60, NxGen MF80	NxGen MF80
Org. Phase	12.5 mM GenVoy-ILM™ in Ethanol			
Aq. Phase	0.174 mg/mL CleanCap® 5moU Epo mRNA in RNA formulation buffer (pH 7.0)			
Total micromixing volume	4 mL	4 mL	20, 25, 55 mL	325 mL
Flow Rate Ratio (FRR) [Org : Aq]	3:1			
Total Flow Rate (TFR)	12 mL/min	12 mL/min	12 mL/min, 60 mL/min & 90 mL/min	200 mL/min
In-line dilution ratio (Buffer: Micromix volume)	-	3:1	3:1, 3:1, 2:1	3:1
Downstream Processing	40X dilution + UF	40X dilution + UF	40X dilution + UF or TFF	40X dilution + UF or TFF

*UF - Ultrafiltration, TFF - Tangential Flow Filtration

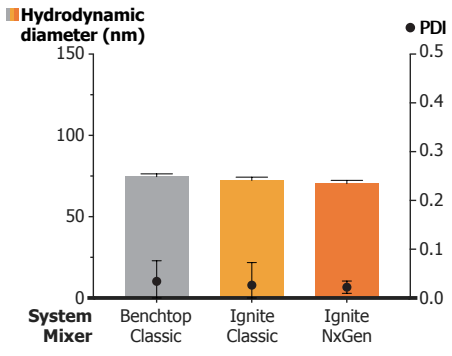
Table 2. TFF Process Parameters for downstream processing of Epo mRNA-LNPs

Process parameters	KrosFlow® Research iII TFF System	GE Äkta™ Flux 6 TFF System
Input Diluted mRNA LNP Volume (mL)	600-800	2000-8000
Sample Reservoir Temperature (°C)	8-12	14-17
Feed Flow Rate (mL/min)	40-130	1500-1700
Expected Shear Rate (s^{-1})	4000	6000
Average TMP (psi)	7	4-5
Average Permeate Flux Rate (LMH)	60	-
Q_p (gm/min)	-	500
Total Run Time (min)	55-63	15-35
Final processed mRNA-LNP Volume (mL)	15-17.5	100-150

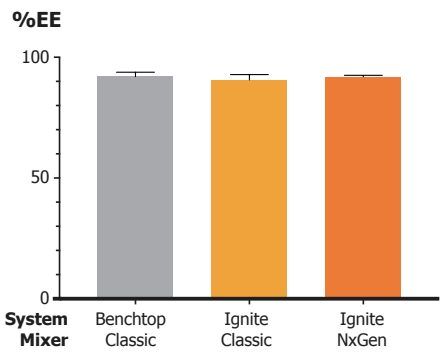
Results

Epo-encoded mRNA-LNP produced with NxGen and Classic mixers on the Ignite system had equivalent size, polydispersity, mRNA encapsulation and in vivo biological activity

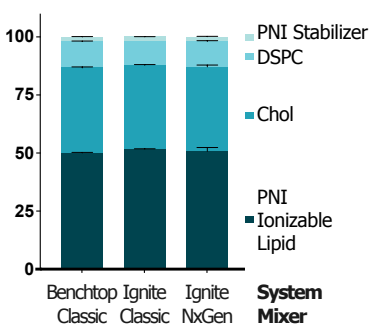
A) Equivalent Size and PDI demonstrated for Classic and NxGen



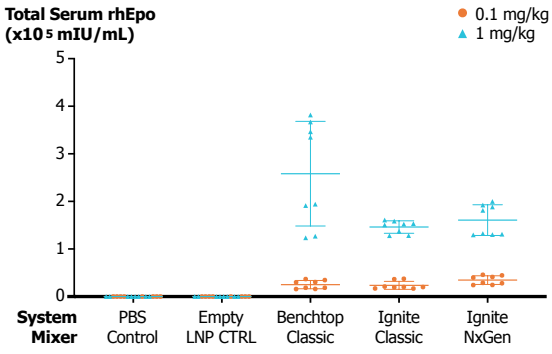
B) Equivalent Encapsulation Demonstrated for Classic and NxGen



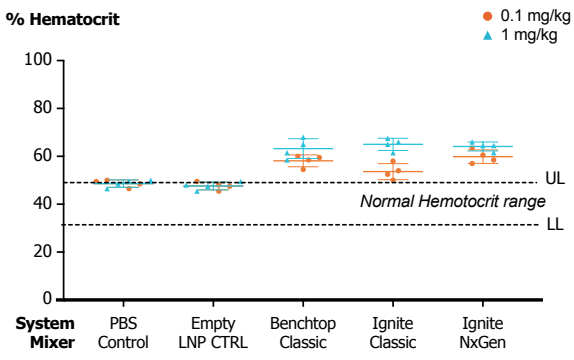
C) Equivalent Composition Demonstrated for Classic and NxGen



D) Comparable Serum Epo Expression demonstrated for Classic and NxGen

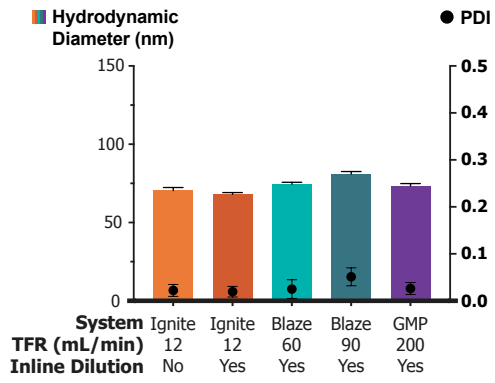


E) Equivalent Elevation of Erythrocyte Levels Demonstrated for Classic and NxGen

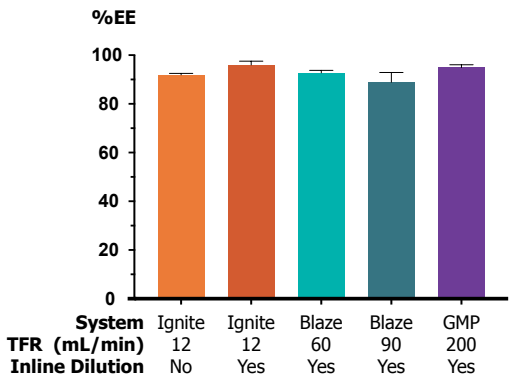


The Epo-encoded mRNA-LNP batch size was scaled by > 80X with NxGen in a single mixer configuration with minimal process optimization

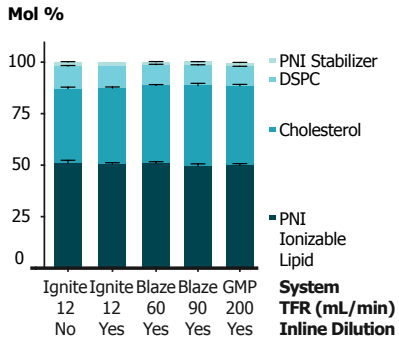
A) Equivalent Size and PDI Achieved Across the Scales and Systems



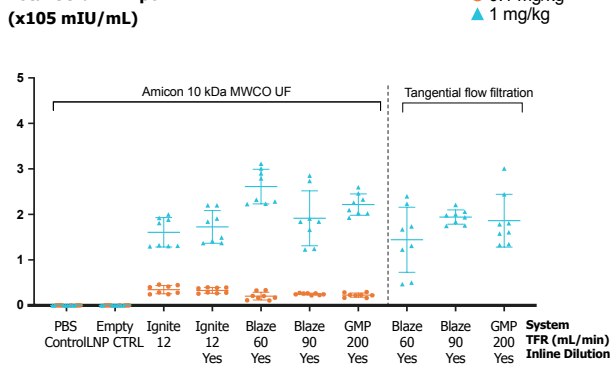
B) Equivalent Encapsulation Achieved Across Scales and Systems



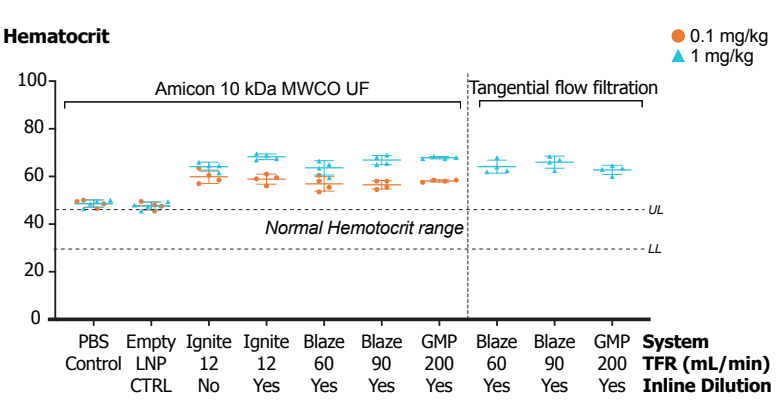
C) Composition maintained Across Scales and Systems



D) Treatment Induced Serum Epo Expression Across Scales



E) Treatment Stimulated Erythrocyte Production Equivalently Across Scales



Conclusions

- NxGen technology enables rapid production and seamless scale-up of nucleic acid LNPs to accelerate drug development from bench-scale discovery research towards clinical-scale GMP manufacturing
- GenVoy-ILM reagent in combination with NxGen manufacturing can be utilized to produce high-quality RNA-LNPs for proof-of-concept and preclinical studies with any therapeutic mRNA