Microfluidic Nanoprecipitation Technique using Novel NxGen™ Technology: A Powerful Tool for Scalable Manufacture of Liposomal and Polymer-based Drug Delivery

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Introduction

Purpose

Showcase the utility, versatility, and scalability of a novel NxGen microfluidic mixer for development and manufacturing of small molecule-loaded drug delivery systems.

Objectives

1. Demonstrate optimization of liposomes and polymer nanoparticles (NPs) with NxGen technology on NanoAssemblr Ignite
2. Optimize size by tuning total flow rate, flow rate ratio, or reagent concentration
3. Demonstrate in-line loading of a clinically-relevant hydrophobic drug in both liposomes and polymer NPs
4. Scale liposome production from Ignite™ to Blaze™ and GMP Systems achieving up to 200 mL/min with a single mixer

Methods

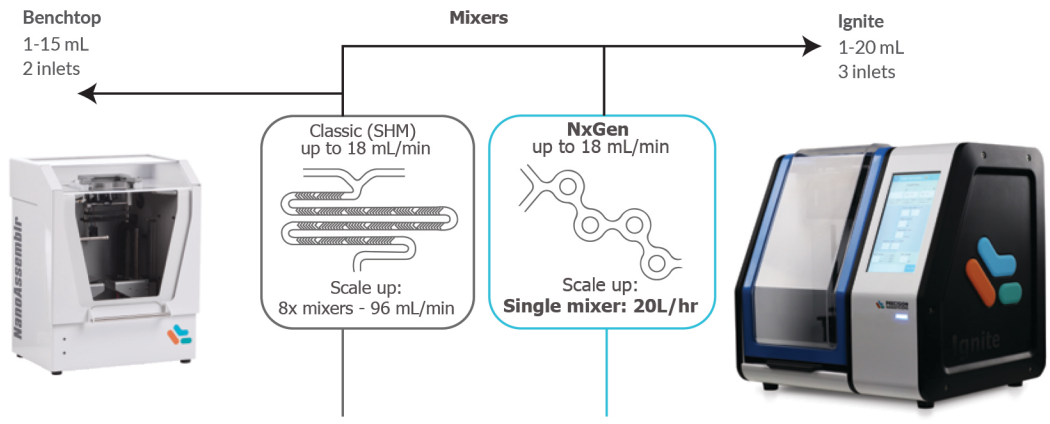
Liposomes: Phospholipids, cholesterol, PEGylated lipid, soy-PC, egg-PC and verteporfin were procured from different vendors. Soy-PC or egg-PC, cholesterol and PEGylated lipid were dissolved in absolute ethanol at a 52:45:3 molar ratio. Calcium and magnesium-free PBS buffer at neutral pH was used as the aqueous phase. To form unilamellar liposomes, the organic and aqueous phases were rapidly mixed using NxGen and Classic mixers at flow rate ratios and total flow rates indicated. Optionally, verteporfin was loaded by dissolving it in the lipid solution at 1 mg/mL (0.09 molar ratio to total lipids) prior to microfluidic mixing with about 2.5% by volume DMF added to ethanol as a co-solvent.

PLGA NPs: PLGA ester-capped polymer (lactide:glycolide 50:50) and MW 45-55 kDa was first solubilized in acetonitrile at concentrations indicated. Optionally, verteporfin dissolved in DMSO at 20 mg/mL was added to the polymer solution at v/v ratio of 1:20. 2% polyvinyl alcohol in deionized water was used as the aqueous phase. To form PLGA nanoparticles, the organic and aqueous phases were rapidly mixed using NxGen and Classic mixers at a 1:1 flow rate ratio and 8 mL/min total flow rate.

Scale Up of Liposomes: POPC and cholesterol dissolved in anhydrous ethanol at 55:45 molar ratio at 16.9 mM were mixed with 1X PBS using the NanoAssemblr® Systems employing Classic or NxGen mixers as indicated. TFR were as indicated and the FRR was set at 3:1. Formulated liposomes were collected and 250 µL of formulate was diluted in 750 µL 1X PBS. 200 µL of diluted liposomes was added to 200 µL of 1X PBS for sizing by dynamic light scattering (Zetasizer Nano ZS, Malvern Panalytical, UK).

Methods Overview

NanoAssemblr® Systems Compared

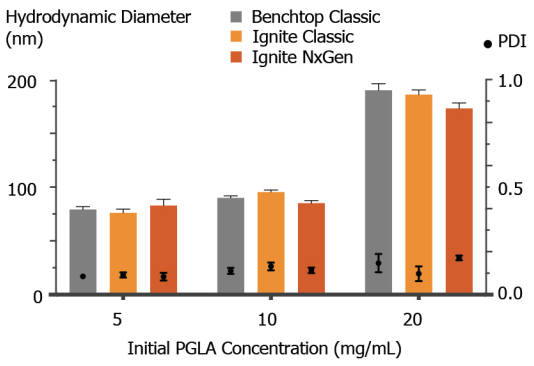


The Classic mixer was the go-to microfluidic technology for drug devekopment that offers many advantages over conventional methods.

NxGen is the evolution of microfluidic nanomedicine manufacturing. It is the only technolgy that can scale nanoparticle production in a single mixer from mL/min to L/h while maintaining time-invariant particle formaton conditions.

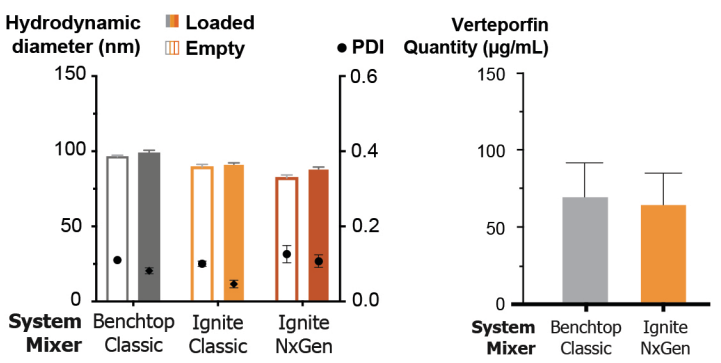
Drug-Loaded PLGA NPs were Developed Using Ignite with NxGen Technology at Bench Scale

Size Systematically Optimized by Concentration



Formulation	PLGA ester capped (50:50), 45-55kDa
Org. Phase	5, 10, or 20 mg/mL PLGA in acetonitrile
Aq. Phase	2% w/v PVA in Milli-Q water
FRR	1:1
TFR	8 mL/min
Replicates	5
Downstream Processing	Dilution, Dialysis

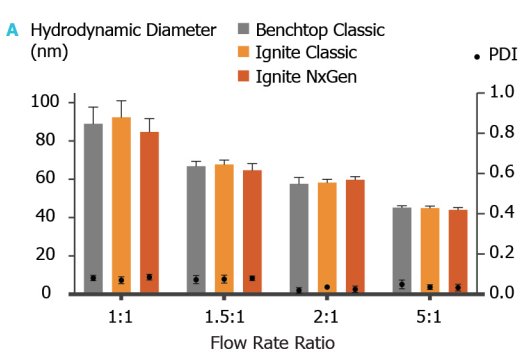
Inline Loading of Verteporfin with Retained Size and Equivalent Drug Encapsulation



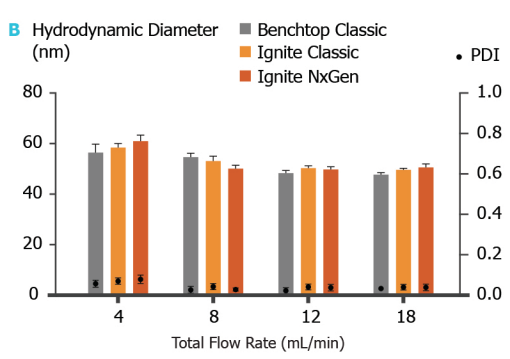
Formulation	PLGA ester capped (50:50), 45-55kDa
Org. Phase	PLGA 10mg/mL + Verteporfin in acetonitrile
Aq. Phase	2% w/v PVA in Milli-Q water
FRR	1:1
TFR	8 mL/min
Replicates	5
Downstream Processing	Dilution, Dialysis

Drug-Loaded Liposomes were Developed Using Ignite with NxGen Technology at Bench Scale and Scaled By Increasing Throughput with Single Mixer up to 16x

Size Systematically Optimized by Total Flow Rate and Ratio

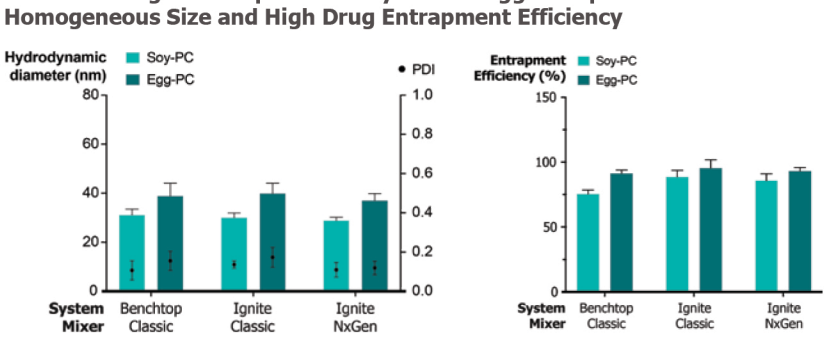


Formulation	POPC/Chol/DSPE-PEG2k (52:45:3 mol%)
Org. Phase	10 mg/mL lipids in ethanol
Aq. Phase	PBS
FRR	A) As indicated B) 1:1



TFR	A) 8 mL/min B) As indicated
Replicates	5
Downstream Processing	Dilution, Dialysis

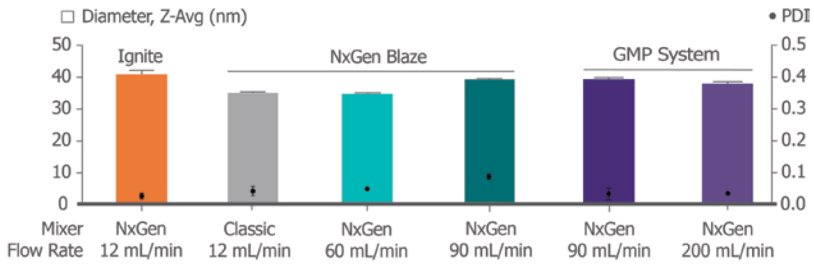
Inline Loading of Verteporfin in Soy-PC and Egg-PC Liposomes with Homogeneous Size and High Drug Entrapment Efficiency



Formulation	PC/Chol/DSPE-PEG2k (52:45:3 mol%)
Org. Phase	10 mg/mL lipids + 1mg/mL verteporfin in ethanol/DMF (90/10)
Aq. Phase	PBS

FRR	2:1
TFR	12 mL/min
Replicates	5
Downstream Processing	Dilution, Dialyis

Liposomes Optimized on Ignite Were Scaled Up to Blaze and GMP Systems up to 200 mL/min Through Single NxGen Mixer



Formulation	PC/Chol (55:45 mol%)
Org. Phase	16.9 mM lipids in Ethanol
Aq. Phase	PBS
FRR	3:1
TFR	As Indicated
Downstream Processing	Dilution

Conclusions

- A NxGen microfluidic mixer for rapid, reproducible, optimization of liposomes and polymer NPs was demonstrated
- Simultaneous drug loading and particle formation was demonstrated for both liposomes and polymer NPs
- The scalability of NxGen technology was demonstrated using a liposome formulation optimized at 12 mL/min at bench scale on Ignite and scaling batch size by increasing single-mixer throughput to 90 mL/min on Blaze and 200 mL/min on the GMP System.
- Overall, these results support the utility of NxGen technology as a robust, scalable manufacturing method for parenteral nanoparticle formulations to treat a wide range of diseases.