Lipid Nanoparticle Production for mRNA Delivery: Impact of Mixing Technologies on Transfection Performance *in vivo*

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PURPOSE

mRNA-loaded lipid nanoparticles (LNPs) have revolutionized vaccines & gene therapy drug products. Two different mixing technologies exist for their production: **jet impinging** and **microfluidics**, whereas the former is preferred in **high-volume production** due to its ease of **scale-up.**

Here, we showcase a **side-by-side comparison** between **jet impinging (LEON equipment) & microfluidic technology** to evaluate the impact of the mixing principle on transfection performance ***in vitro* & *in vivo*** experiments. A clinically relevant lipid composition loaded with firefly luciferase (FLuc) mRNA as reporter system was used.

METHODS

- **LEON Equipment train:**

Equipment	Details	Volume output [ml/min]	Footprint (H x W x D) [m]
LEON 1	batchwise production	Bench scale: 1-80	1 x 1 x 0.5
LEON 2	continuous flow ready	Bench scale: 1-80	1 x 1 x 0.5
LEON 3	continuous flow ready	Pilot scale: 30-500	1 x 1 x 0.6
LEON GMP unit	continuous flow ready	Commercial scale: 300+	2 x 0.8 x 1.2

- **Formulation:** Onpattro® lipid composition: DLin-MC3/Cholesterol/DSPC/DMG-PEG2000 – 50/38.5/10/1.5 mol% with total lipid concentration of 10 mg/ml. FLuc mRNA (Trilink or APExBIO) concentration of 0.09 mg/ml in non-solvent.
- **LNP production:** LNPs were produced with a flow rate ratio (FRR) of 3:1 (non-solvent:solvent) and total flow rate (TFR) = 30 ml/min using an impinging jet reactor setup (LEON 1) and TFR 10 ml/min using a microfluidics setup (Ignite™, Precision NanoSystems). Lipids were dissolved in EtOH (solvent) and precipitated against 50 mM citrate buffer (pH 4) (non-solvent). Samples were diluted, subjected to dialysis overnight (PBS pH 7.4) and concentrated to approx. 1 mg/ml.
- **Particle characterization:** Particle size & polydispersity index (PDI) were determined by dynamic light scattering (DLS) using a Stunner instrument (Unchained Labs). Cryo-transmission electron microscope (TEM) samples were inspected on a Tecnai F20 TEM.
- **Encapsulation efficiency:** Encapsulated mRNA was quantified using a fluorogenic Quant-iT™ RiboGreen™ RNA assay kit (Thermo Fisher Scientific).
- ***In vitro* transfection assay:** HepG2 cells were incubated in a 24-well plate format with various doses of FLuc mRNA-loaded LNPs.
- ***In vivo* transfection assay:** B6 albino mice (n=6 per group) received a single IV (tail vain) injection of 60 µg mRNA-loaded LNPs (2.4 mg/kg). Bioluminescence imaging on an IVIS Spectrum imaging system (PerkinElmer) was performed 6 h, 24 h & 48 h after LNP administration.

RESULTS

- Both mixing technologies generated FLuc-mRNA loaded LNPs according to cryo-TEM (Figure 1) with **comparable size, PDI (Figure 2)** and **encapsulation efficiency (EE%) (Figure 3).**

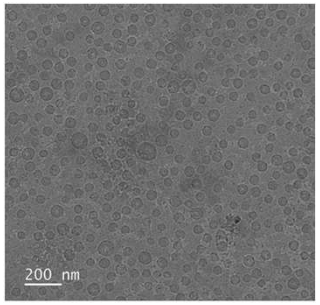
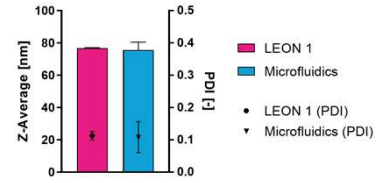
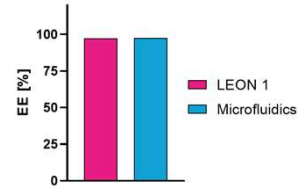


Figure 1. Cryo-TEM image of a sample of FLuc mRNA-loaded LNPs produced with LEON's impinging jet technology showing spherical shaped particles.



Equipment	Avg. Z-Average [nm]	Avg. PDI [-]
LEON 1	76.8	0.11
Microfluidics	75.6	0.11

Figure 2. Particle size (Z-average) & polydispersity index (PDI) of LNP samples analyzed by dynamic light scattering (DLS).



Equipment	EE%
LEON 1	97.5
Microfluidics	97.7

Figure 3. Encapsulation efficiency (EE%) of LNP samples determined by a fluorometric RiboGreen assay.

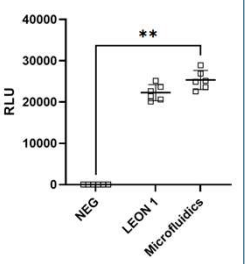


Figure 4. *In vitro* transfection assay using 10 µg mRNA (according to EE% assay) per transfection. All data n=6, non-parametric, Kruskal-Wallis post AVG+/-SD, ** p<0.01.

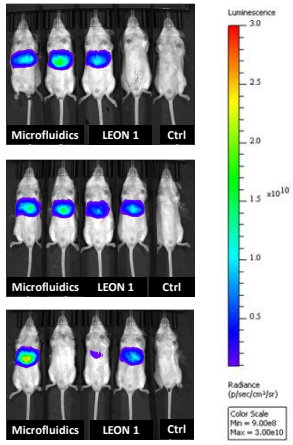


Figure 5. *In vivo* gene delivery as observed by luciferase bioluminescence imaging.

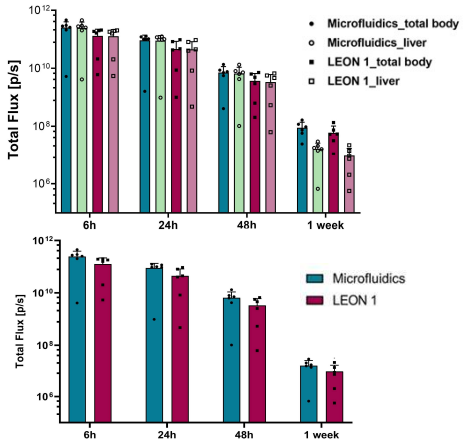


Figure 6. *In vivo* gene delivery quantification for total body and liver (top) and total body (bottom) over time.

- The high encapsulation efficiency **(EE% > 97%)** of samples of both mixing technologies translated into high transfection rates in cell assays (Figure 4) and *in vivo* (Figure 5 & 6). FLuc expression seemed to peak at 6h post dosing and declined thereafter. In vivo imaging suggests **systemic delivery of LNPs** in both groups (LEON 1 and microfluidics) and predominant transfection of the liver as target organ. **5/6 animals positive** in both groups.

CONCLUSIONS

- ✓ **LEON equipment efficiently** enables the production of mRNA-loaded LNPs with **low PDI** (PDI < 0.12) and **high encapsulation efficiency** (EE% > 97%).
- ✓ LEON equipment enables the production of mRNA-loaded LNPs under low endotoxin burden RNase contamination.
- ✓ LNPs produced with LEON 1 equipment **under turbulent mixing conditions** (TFR: 30 ml/min) were **comparable** to LNPs produced **with a laminar flow-based system** (Ignite, Precision NanoSystems) in terms of:
  - **Size & PDI (72-79 nm, PDI < 0.12)**
  - **Encapsulation efficiency** (EE% > 97%).
  - ***In vitro* & *in vivo* transfection established** - non-inferiority of LEON 1 vs. microfluidics produced mRNA/LNPs demonstrated based on expression yields and kinetics.
  - Further studies with higher flow rates (TFR > 300 ml/min) are currently on-going for high volume manufacturing of LNPs as part of LEON's database.

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