# Lipid Nanoparticle Production for mRNA Delivery: Comparison of Different Turbulent Mixing Technologies


**Vogler J.**

**leon-nanodrugs GmbH, Munich, Germany**

**WEBSITE: www.leon-nanodrugs.com**
**EMAIL: j.vogler@leon-nanodrugs.com**


## PURPOSE

**mRNA-loaded lipid nanoparticles (LNPs)** have revolutionized vaccines & gene therapy drug products. Turbulent mixing has proven to be the preferred manufacturing technology for high-volume production due to its ease of scale-up compared to e.g. microfluidics.

Here, we show **comparative data on jet impinging technology from LEON vs. conventional T-junction mixing** to evaluate the impact of the mixing technology on the particle properties and *in vivo* tranfection efficiency. A clinically relevant lipid composition loaded with polyA surrogate or firefly luciferase (FLuc) mRNA as reporter system was used.

## METHODS

- **Mixing technologies:**

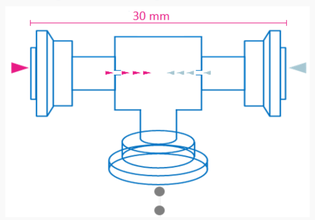


**Figure 1. LEON impinging jet mixer**

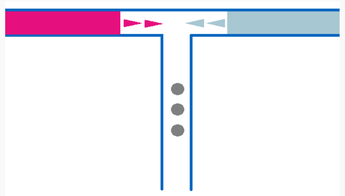

**Figure 2. Conventional T-junction mixer**

- **LNP production:** LNPs were produced by mixing a lipid solution consisting of ionizable cationic lipid/Cholesterol/DSPC/PEG-Lipid in ethanol with a buffered aqueous solution containing polyA or FLuc mRNA. A flow rate ratio (FRR) of 2:1, 3:1 and 4:1 as well as a total flow rate (TFR) of 40 ml/min and 80 ml/min was selected for the manufacturing of polyA loaded LNPs using the LEON impinging jet mixer. Results were compared with LNPs produced with a FRR of 3:1 and TFR of 40 ml/min using a conventional T-junction mixer. Subsequently Fluc mRNA LNPs were manufactured at a FRR of 3:1 and TFR of 40 ml/min with both LEON impinging jet mixer and conventional T-junction mixer. All samples were dialyzed against PBS pH 7.4 without prior dilution.
- **Particle characterization:** Particle size & poly dispersity index (PDI) were determined by dynamic light scattering (DLS) using a Malvern Zetasizer (Zetasizer Nano ZS, Malvern, UK).
- **Encapsulation efficiency:** Encapsulated mRNA was quantified using a fluorogenic RiboGreen™ RNA assay kit.
- ***In vivo* transfection assay:** 5 mice each received a single IV (tail vain) injection of 0.3 mg/kg or 1 mg/kg mRNA-loaded LNPs produced with the LEON impinging jet reactor or a conventional T-junction mixer. Bioluminescence of liver homogenates was determined 4 h after LNP administration.

## RESULTS

- Both mixing technologies generated polyA and FLuc-mRNA loaded LNPs with **comparable size, PDI, encapsulation efficiency (EE%) (Table 1), morphology (Figure 3) and *in vivo* activity (Figure 2).**

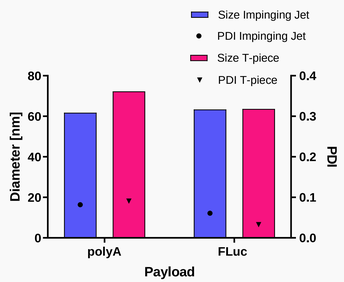


**Figure 1.** Size (z-average) and PDI of polyA and FLuc mRNA loaded LNPs measured by DLS post dialysis. Particles were produced at FRR=3:1 and TFR=40 ml/min with both LEON impinging jet reactor and conventional T-piece mixer.

**Table 1.** Size (z-average), PDI and encapsulation efficiency (EE%) of polyA and FLuc mRNA loaded LNPs produced with different process parameters. Results for particles produced with a conventional T-piece are highlighted in pink.

| Sample | Process Step | Size (Z-Ave nm) | PDI | EE% | TFR (ml/min) | FRR | DS |
|---|---|---|---|---|---|---|---|
| Sample A (Impinging Jet) | Post-dialysis | 71.6 | 0.093 | 95 | 40 | 2:1 | Poly-A |
| Sample B (Impinging Jet) | **Post-dialysis** | **61.78** | **0.082** | **97** | **40** | **3:1** | |
| Sample C (Impinging Jet) | Post-dialysis | 71.97 | 0.157 | 97 | 40 | 4:1 | |
| Sample D (Impinging Jet) | Post-dialysis | 62.01 | 0.103 | 98 | 80 | 3:1 | |
| Sample E (T-piece) | **Post-dialysis** | **72.33** | **0.091** | **99** | **40** | **3:1** | |
| Sample F (Impinging Jet) | Post-dialysis | 63.35 | 0.061 | 98 | 40 | 3:1 | FLuc |
| Sample G (T-piece) | Post-dialysis | 63.65 | 0.033 | ND | 40 | 3:1 | |
| Sample H (Impinging Jet) | Post-conc. | 65.68 | 0.007 | 99 | 40 | 3:1 | |
| Sample I (T-piece) | Post-conc. | 64.23 | 0.034 | 99 | 40 | 3:1 | |

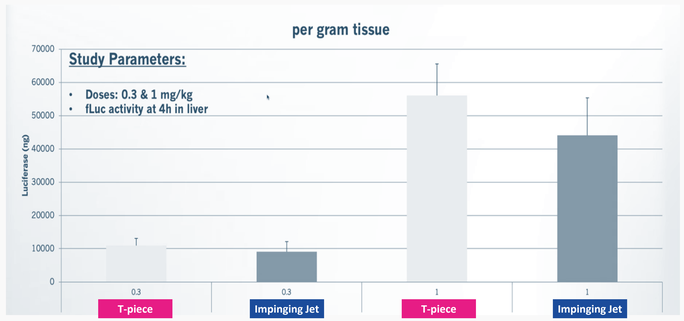


**Figure 2.** *In vivo* transfection assay. Bioluminescence of liver homogenates was determined 4 h after IV injection with FLuc mRNA loaded LNPs produced with LEON impinging jet reactor or a conventional T-piece. n=5

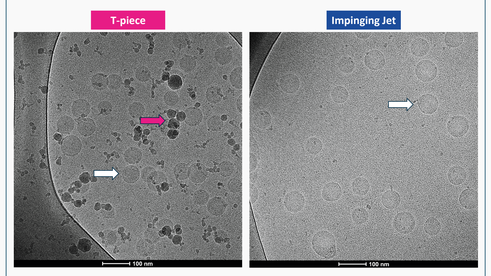


**Figure 3.** Cryo-TEM images of FLuc mRNA loaded LNPs produced with LEON impinging jet reactor or conventional T-piece. White arrows indicate spherical LNPs. Pink arrows indicate spheroid frost which are sample preparation artifacts and not indicative of LNP characteristics.

- The variation of FRR for polyA loaded LNPs produced with LEON impinging jet reactor showed little impact on the particle properties with FRR=3:1 resulting in the smallest size and PDI. Increasing the TFR from 40 ml/min to 80 ml/min did not result in a substantial change in size and PDI when using the LEON impinging jet reactor.
- Both mixing technologies result in homogenous spherical particles according to cryo-TEM analysis.

## CONCLUSIONS

- ✓ **LEON's impinging jet reactor** technology enables the manufacturing of mRNA loaded LNPs with **small size, low PDI** and **high encapsulation efficiency.**
- ✓ LNPs produced with LEON's impinging jet reactor technology are **comparable** with LNPs produced with a conventional T-piece mixer in terms of **particle size, PDI, EE%, morphology, and *in vivo* activity.**
- ✓ **LNP characteristics** (size, PDI, EE%) of particles produced with LEON's impinging jet reactor technology are **consistent over a wide range of total flow rates** (40 – 80 ml/min tested).
- ✓ The collected **scientific data demonstrate** once again the **quality** of LEON's process technology to support **product design and development** based on LEON's expertise and its **data driven approach**.


## ACKNOWLEDGMENT

We thank Acuitas Therapeutics (Vancouver) for the collaborative support on the present work.


ENABLING NANO NOW