Simplifying Progress

Stability of mRNA and Lipid Nanoparticles Stored in Single-Use Bags

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Summary

RNA therapy holds immense promise in revolutionizing the treatment landscape for various diseases. For more effective delivery of mRNA to cells, mRNA is encapsulated in lipid nanoparticles (LNPs). During the manufacturing process, mRNA and LNPs need to be stored in storage bags in an ultra-low freezer. In this presentation, we studied the stability of mRNA and mRNA-encapsulated LNPs stored in single-use bags. We prepared LNPs using our lab-made microfluidics mixing device, which enables us to prepare LNPs at a rate as fast as 120 mL/min. We report that mRNA-loaded LNPs were stable for at least 6 months at -80°C in Flexboy® bags and that in vitro transfection activity was confirmed using the Incucyte® AI Cell Health Analysis Software Module. We also found that the count rate for dynamic light scattering (DLS) dropped within the first hour of storage, suggesting potential particle adsorption to the storage container. The stability of mRNA and mRNA-encapsulated liposomes (in vivo-jetRNA®+) was also reported.

Flexboy® and Flexsafe® Bags

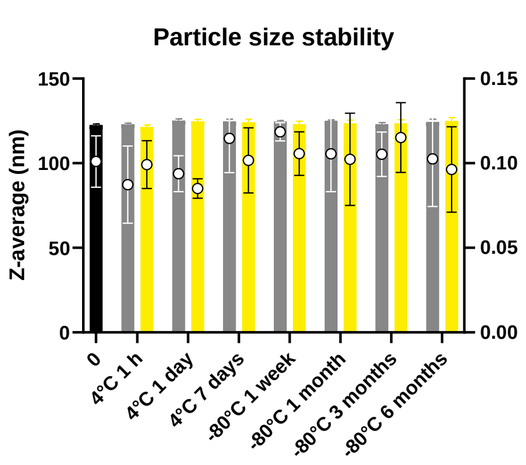
- Flexboy® bags (5 mL to 50 L) and Flexsafe® 2D bags (20 mL to 50 L) have provided robust solutions for the storage, sampling, and transfer of all biopharmaceutical fluids in all process steps.
- These bags promise quality supply, robustness, closure integrity, biocompatibility, and cell growth performance.

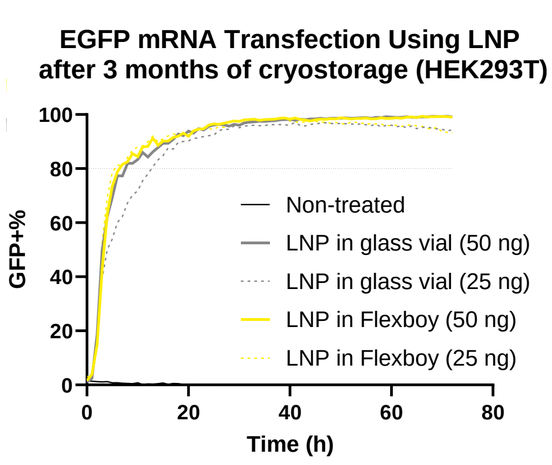
Flexboy® bags

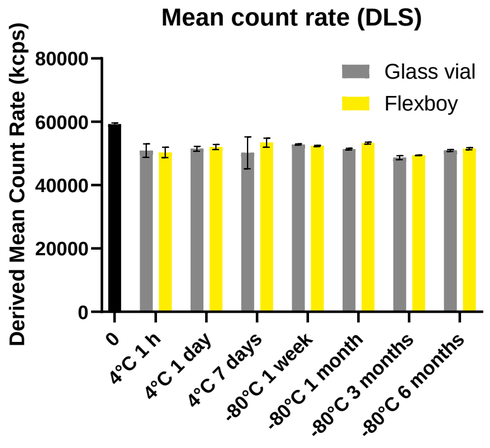
Flexsafe® bags

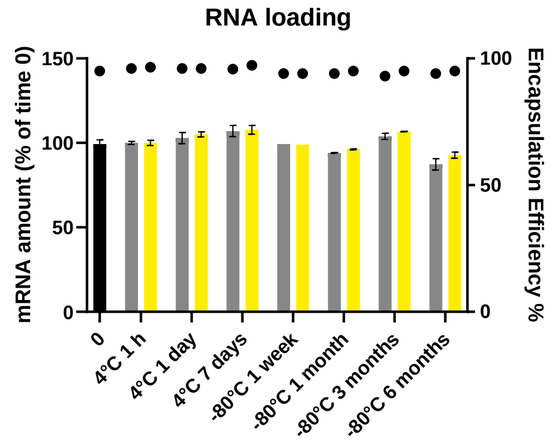
LNP Stability in Flexboy® Bags

- Confirmed stability of LNP in Flexboy® bags at -80°C at least for 6 months.
- Flexboy is suitable for cryostorage of LNP.
- Observed drop of count rate by ~15% in the first hour, suggesting potential particle adsorption to the storage containers.



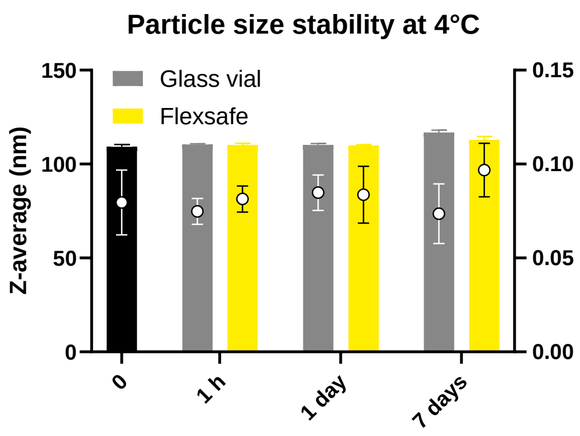


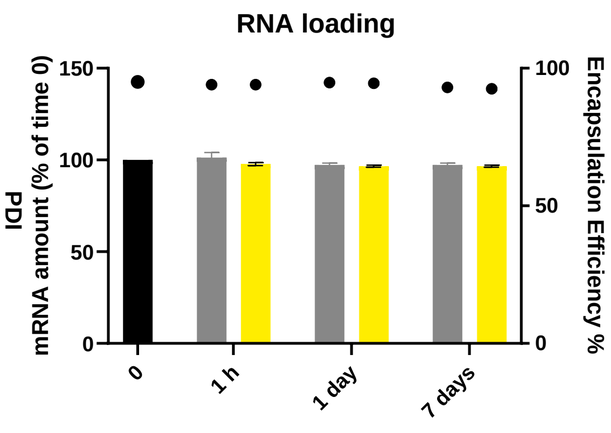




LNP Stability in Flexsafe® Bags

- Confirmed stability of LNP in Flexsafe® bags at 4°C at least for 1 week.



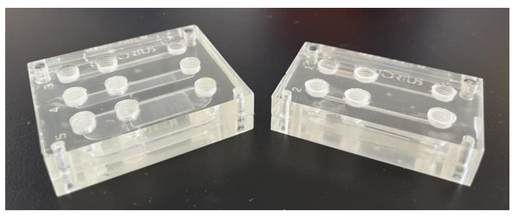


Materials and Methods

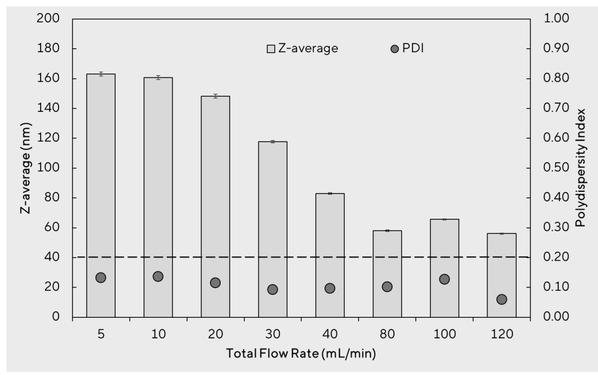
- LNP, made of ALC-0315, cholesterol, DSPC, and ALC-0159 lipids, was prepared using a microfluidic device. EGFP mRNA was encapsulated in the LNP. Sample was dialyzed against Tris buffer with 10% sucrose and then sterile filtered. Particle size and count rate were measured using Zetasizer, and mRNA loading was measured using RiboGreen assay.
- Transfection assay: Fluorescent images were taken using IncuCyte® S3 and analyzed using IncuCyte® AI Cell Health Analysis Software Module.

Membrane Microfluidics for Nanoparticle Synthesis

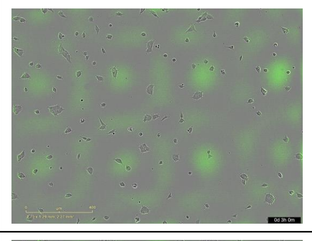
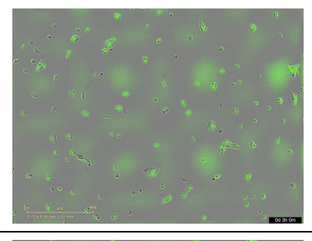
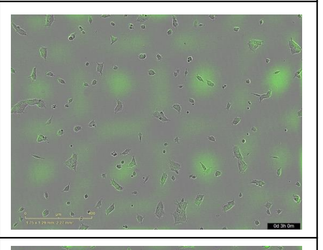
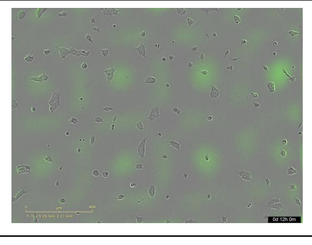
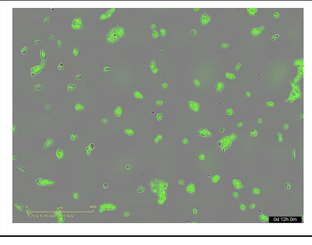
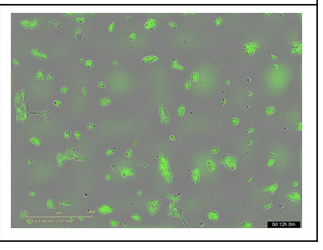
- Membrane chip consisting of micro-channels, track-etched membrane, and pressure-sensitive adhesives.
- Membrane pore size and mixing channel width can be tailored as needed.
- Scalable (up to 120 mL/min) production of LNP with consistent critical quality attributes (CQAs).



Membrane mixer chip

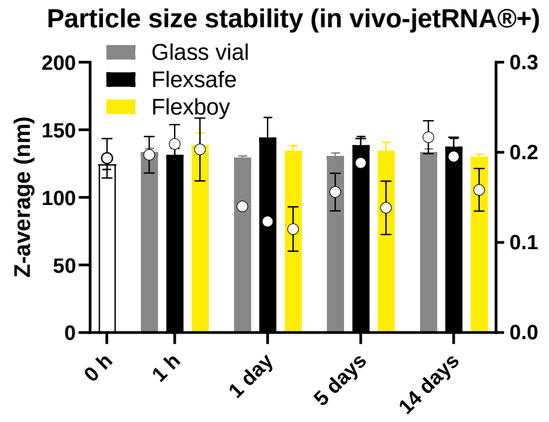


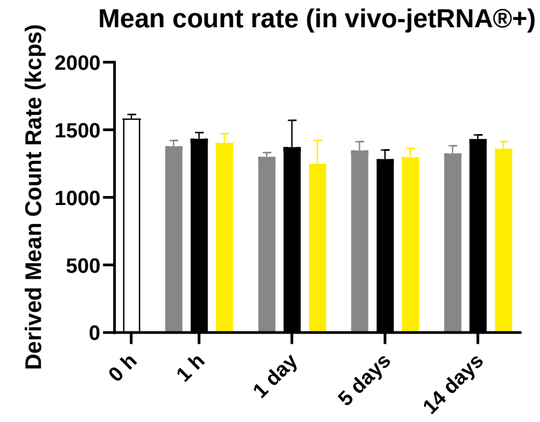
Transfection assay after 3-month storage of LNP at -80°C (HEK293T cell).

	Non-treated	LNP in Flexboy (25 ng RNA)	LNP in Glass Vial (25 ng RNA)
3 h			
12 h			

Stability of *in vivo*-jetRNA®+ in Storage Bags

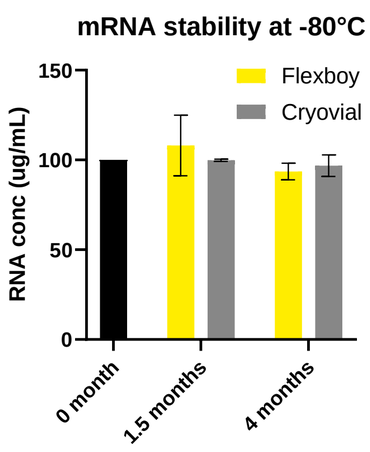
- Confirmed stability of mRNA-encapsulated in vivo-jetRNA®+ in Flexboy® and Flexsafe® bags at 4°C at least for 2 weeks.
- Observed drop of count rate by ~15% in the first hour, suggesting potential particle adsorption to the storage containers.

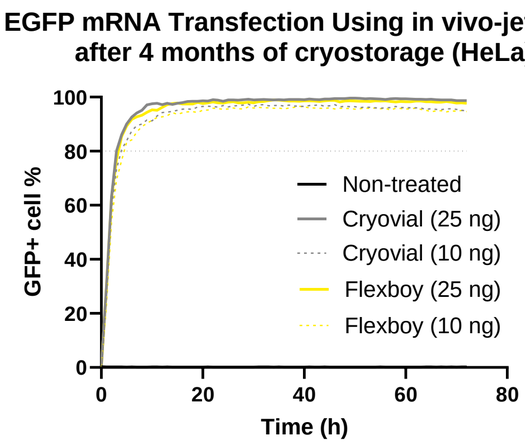




mRNA Storage in Flexboy® Bags

- Confirmed stability of mRNA in Flexboy at -80°C at least for 4 months.
- Flexboy is suitable for cryostorage of mRNA.
- Adsorption of mRNA to the storage containers was not observed.





IncuCyte® S3