Effective screening of LNP formulations with centrifugal microfluidics devices

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KEY FINDING

Plasmid DNA can be delivered by LNPs formulated using centrifugal microfluidics devices.

ABSTRACT

Lipid nanoparticles (LNPs) have emerged recently as an extremely effective method for delivering nucleic acids to cells for a variety of therapeutic purposes. With 4-5 lipid components each and hundreds of published lipids for incorporation, development of optimized efficacious formulations can be an onerous task. Rapid and inexpensive testing of LNP formulations has largely been accomplished using hand-mixing of the components with a typical laboratory pipette. While this method does generate encapsulated cargo, the user-to-user variability and resulting larger particles with higher polydispersity make it less effective and scalable than microfluidics-based methods. We tested single-use centrifugation-based microfluidics devices for screening several LNP formulations and found this method to be rapid and reliable.[1] Using these devices, we evaluated standard mRNA formulations as well as the incorporation of 9(10)-nitrooleic acid (NOA) and DOTAP into pDNA cargo formulations. We evaluated the biophysical characteristics of the resulting LNPs, as well as the effects of the different formulations on cells, from transfection to viability and immunogenicity. The increased efficiency of formulation and the decrease in required resources (notably mRNA cargo) allowed more formulations to be tested in less time. Of the formulations tested, the standard mRNA LNPs behaved similarly to LNPs made using more traditional microfluidics methods. pDNA-containing LNPs were also successful mediators of transfection, though some cytotoxicity was observed. Application of these methods to screening of formulations promises to enhance the speed of innovation in the field and lower the barrier to entry for researchers interested in applying LNPs to their research problem.

RESULTS

	ALC-0315 (Opt pDNA)	ALC-0315 (Opt pDNA+NOA)	ALC-0315	SM-102
Ionizable Lipid	40	32	40	50
Cholesterol	46.4	37.1	46.4	38.5
DSPC	-	-	-	10
DOTAP	12.1	9.7	12.1	-
DMG-PEG2K	-	-	-	1.5
ALC-0159	1.5	1.2	1.5	-
NOA	-	20	-	-
Nucleic Acid	pDNA	pDNA	mRNA	mRNA

TABLE 1 – Composition of LNPs.
LNPs based on the noted lipids were formulated first with either plasmid DNA encoding eGFP (pDNA) or eGFP mRNA. Percent molar ratios of lipid components are shown in the table above.

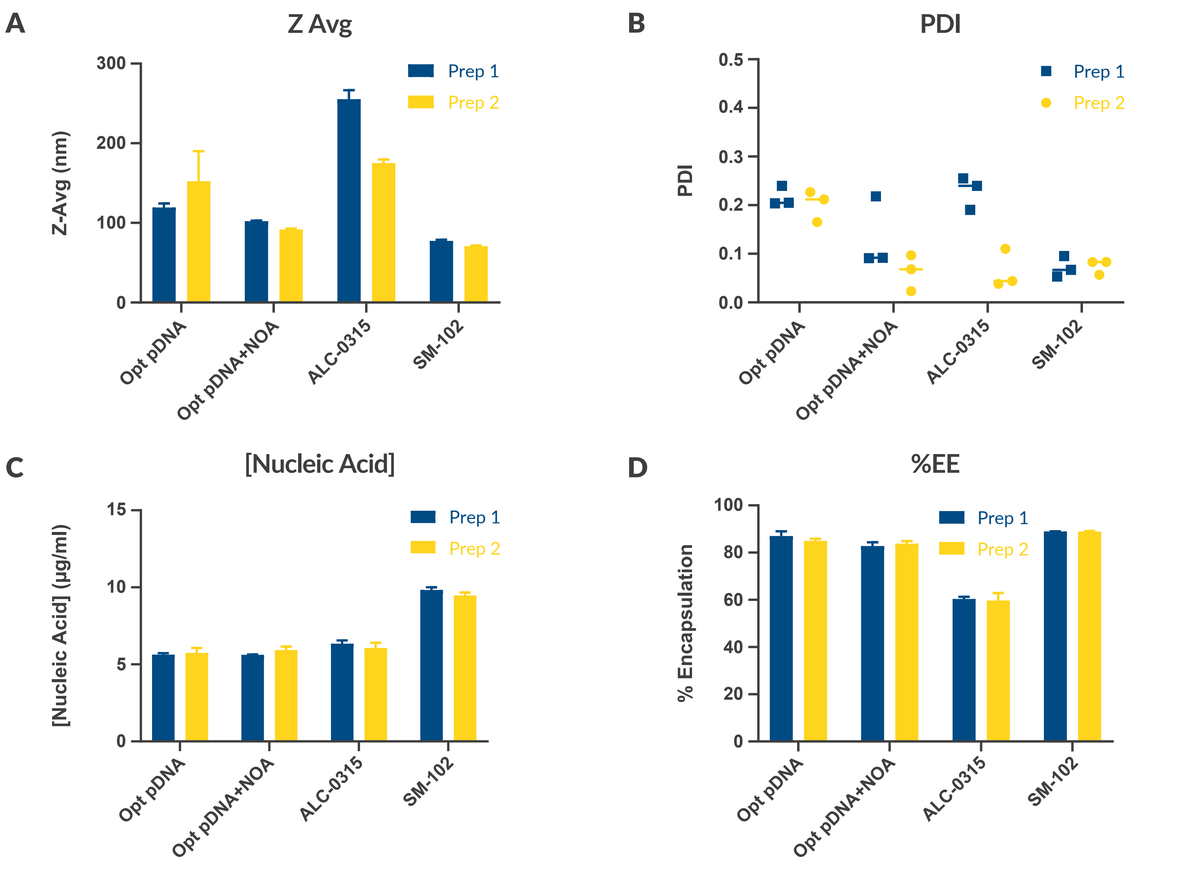

FIGURE 1 – Biophysical characterization of LNPs.
LNPs based on the noted lipids were formulated with either pDNA or eGFP mRNA in duplicate (Prep 1 and Prep 2). Diameter **(A)** and polydispersity index (PDI, **B**) were measured by dynamic light scattering. Nucleic acid concentration **(C)** and encapsulation percentage (%EE, **D**) were measured using a fluorescent assay.

RESULTS

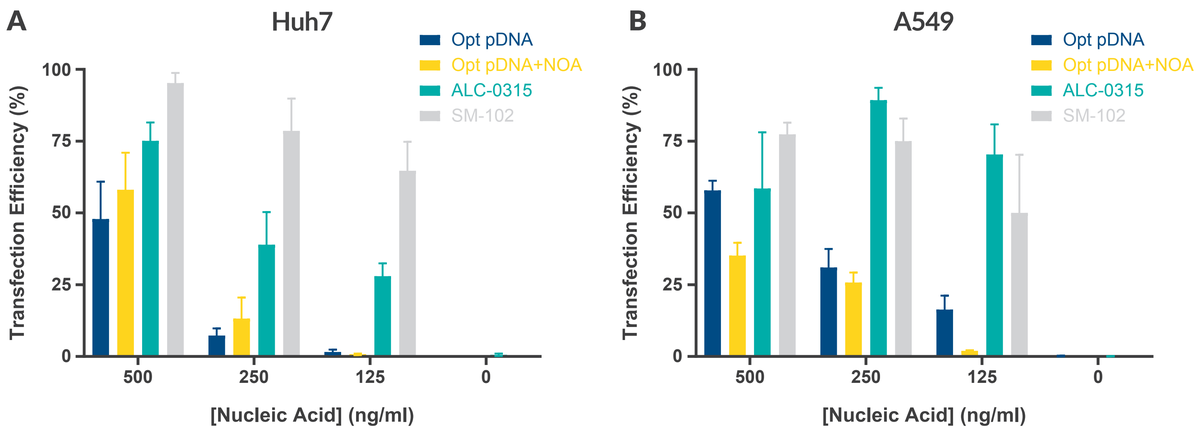

FIGURE 2 – Transfection efficiency of LNPs.
Each LNP and its duplicate was used to transfect hepatocytes (Huh7, left) and lung epithelial cells (A549, right) for 24 hours in a 96-well plate (n=6, per ionizable lipid). Nuclei were stained with Hoechst and transfection efficiency was determined by percent of nuclei which were positive for GFP using an imaging plate reader.

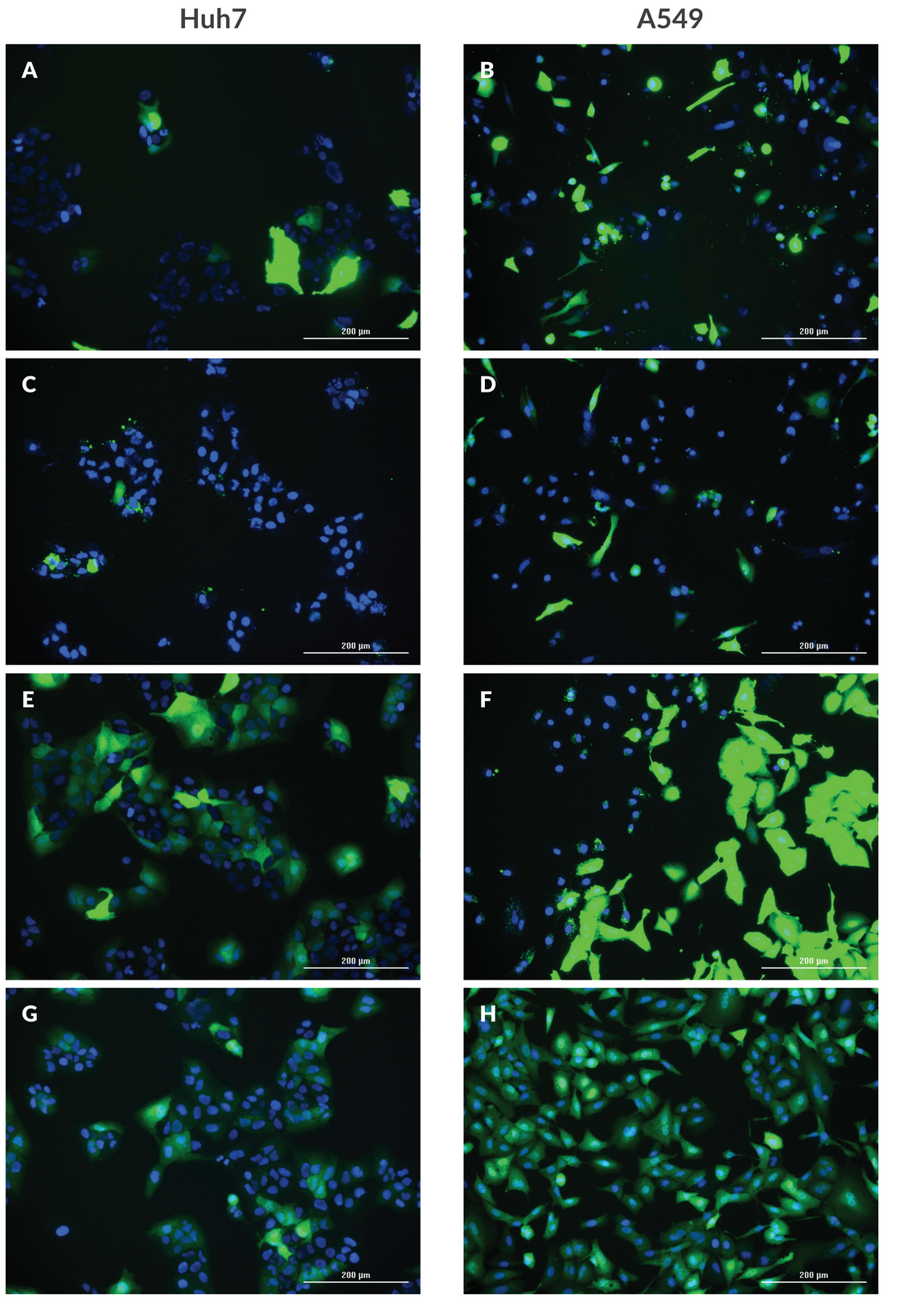

FIGURE 3 – LNPs transfected cells with varying efficiency.
Left column depicts representative images from Huh7 hepatocytes, right column shows A549 lung epithelial cells. Transfections with Opt pDNA **(A, B)**, Opt pDNA+NOA **(C, D)**, ALC-0315 **(E, F)** and SM-102 **(G, H)**. All images shown are from wells transfected with 500 ng/ml of nucleic acid.

CONCLUSIONS

- Centrifugal microfluidics devices produced SM-102-based mRNA LNPs with characteristics similar to those produced with chip-based microfluidics.
- A variety of formulations and cargo types are compatible with centrifugal microfluidics.
- Greater efficiency and lower resource demands make centrifugal microfluidics devices potentially valuable tools for formulation screening.

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References

1. Osem Fluidics, Inc., *prototype in development*, Los Angeles, CA, USA, 2024.
2. Patel, Manthan N. *et al. BioRxiv* [Preprint] 2024. Available from: https://doi.org/10.1101/2024.06.11.598533