Low Volume Production Of Nanoparticles That Are Effective Transfection Systems In iPSC-derived Cells, Immune Cells and Other Primary Cell Cultures

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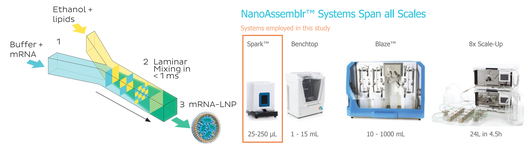
Purpose and Objectives

- Nucleic acid therapies permit access to previously undruggable pathways to treat or potentially cure a wide range of diseases, and lipid nanoparticles (LNPs) are the most clinically advanced non-viral delivery systems
- Significant need exists for robust manufacturing of LNPs at all scales, particularly at very low volumes to enable efficient screening of lipid components and nucleic acid payloads at earlier stages of drug development
- Here, we demonstrate the use of a low volume microfluidic device that provides consistent, controlled formulation conditions for encapsulating just tens of micrograms of mRNA and plasmids into LNPs
- LNP materials were systematically screened for size, polydispersity index, and activity in clinically relevant induced pluripotent stem cell (iPSC)-derived neuroprogenitor cells (NPCs) and cortical neurons

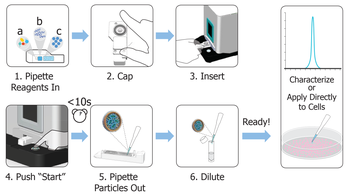
Methods

Microfludic Nanoparticle Production

The NanoAssemblr™ platform uses microfluidics to precisely control the manufacturing of nanoparticles from microlitre to litre scales.

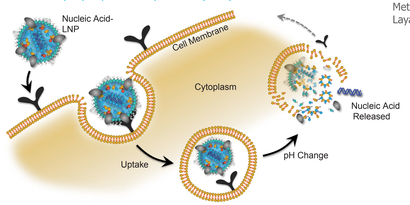


The NanoAssemblr Spark Workflow



- Formulations were rapidly produced with minimum consumption of valuable mRNA and plasmid
- Formulation volumes were < 100µL and required only 12-25 µg of nucleic acid
- Formulations were produced in sterile hood and immediately characterized or applied directly to iPSC-derived cells in culture

Gene Delivery by Lipid Nanoparticle (LNP)

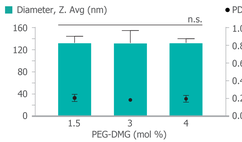


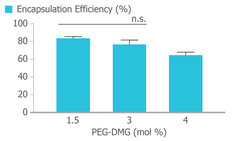
Detailed Materials & Methods: Scan with Layar app.

- Uptake of LNPs occurs via low density lipoprotein receptor mediated endocytosis. Ionizable cationic lipids mediate endosomal disruption and release of nucleic acid
- LNPs were tested for expression in clinically relevant iPSC-derived NPCs and cortical neurons

Screening microgram mRNA formulations

Highest PEG concentration reduces encapsulation efficiency





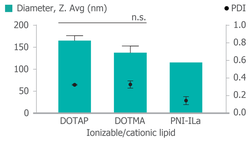
Formulation Details

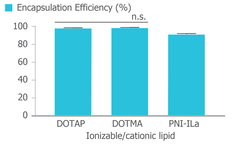
Lipid Composition	PNI-ILa:DSPC:Cholesterol:PEG-DMG 50:10:40-x:x | x = 1.5, 3, 5 mol%
Init. Lipid Conc.	50 mM
mRNA	GFP (996 nt), 0.025 mg
N/P Ratio	4
Organic Solvent	Ethanol
Aqueous Phase	0.695 mg/mL mRNA in 100mM Sodium Acetate
Instrument Setting	3x
Downstream Processing	4x Dilution in nuclease-free PBS

Further Details

Size measured by DLS and Encapsulation Efficiency by Ribogreen assay. Values represent the mean of triplicate formulations. Error bars are the standard deviation. Means grouped by a horizontal line are not significantly different (Tukey's multiple comparison test, P>0.05).

Ionizable cationic lipid produces LNPs with lower size and PDI





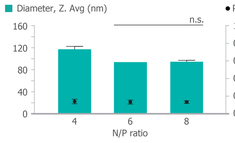
Formulation Details

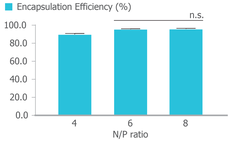
Lipid Composition	CL:DSPC:Cholesterol:PEG-DMG 50:10:48.5:1.5 mol% CL = cationic lipid as labeled on x-axis
Init. Lipid Conc.	50 mM
mRNA	GFP (996 nt), 0.025 mg
N/P Ratio	4
Organic Solvent	Ethanol
Aqueous Phase	0.695 mg/mL mRNA in 100mM Sodium Acetate
Instrument Setting	3x
Downstream Processing	4x Dilution in nuclease-free PBS

Further Details

Size measured by DLS and Encapsulation Efficiency by Ribogreen assay. Values represent the mean of triplicate formulations. Error bars are the standard deviation and are not drawn when error is less than the size of the symbol. Means grouped by a horizontal line are not significantly different (Tukey's multiple comparison test, P>0.05).

Encapsulation efficiency and size reach threshold at N/P ≥ 6





Formulation Details

Lipid Composition	PNI-ILa:DSPC:Cholesterol:PEG-DMG 50:10:38.5:1.5 mol%		
Init. Lipid Conc.	50 mM		
N/P Ratio	4	6	8
mRNA	GFP (996nt)		
Mass (mg)	0.025	0.0167	0.0125
Conc. (mg/mL)	0.695	0.464	0.347
Aqueous Phase	mRNA in 100 mM Sodium Acetate		
Organic Solvent	Ethanol		
Instrument Setting	3x		
Downstream Processing	4x Dilution in nuclease-free PBS		

Further Details

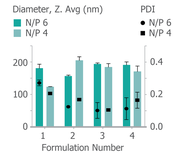
Size measured by DLS and Encapsulation Efficiency by Ribogreen assay. Values represent the mean of triplicate formulations. Error bars are the standard deviation and are not drawn when error is less than the size of the symbol. Means grouped by a horizontal line are not significantly different (Tukey's multiple comparison test, P>0.05).

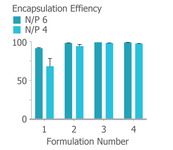
Case Study: Optimizing LNP formulations for plasmid expression in iPSCs

Formulations with similar properties perform differently in iPSC-derrived NPCs undergoing differentiation: Biological activity is a necessary screen

A Physical properties similar between formulations with different lipids and N/P ratios

- A panel of formulations containing PNI-ILa with different helper lipids was created at two N/P ratios
- Formulations 2, 3, and 4 have similar properties
- Panel was created rapidly with the Spark at µg quantities with encapsulation efficiency > 80%

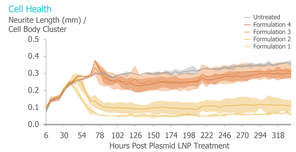


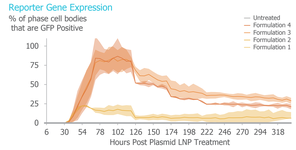


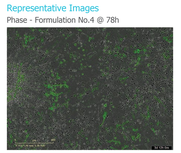
Further Details

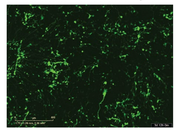
Size measured by DLS and Encapsulation Efficiency by Ribogreen assay. Values represent the mean of duplicate formulations. Error bars are the standard deviation and are not drawn where error is smaller than the symbol.

B Formulations 3 & 4 Exhibit the Least Impact on Neurite Health and The Highest Reporter Gene Expression









- Formulation composition affects neuronal development: formulations 1 and 2 exhibit large decrease in neurite length that is not recovered over time
- Reporter gene expression peaks ~78h after treatment and appreciable expression is still present 14d following treatment
- The same formulations that do not impact neurite length also display high GFP expression, with approximately 80% of cell bodies expressing GFP by 78 hours

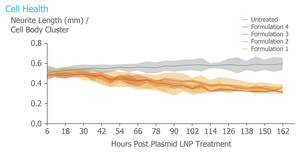
Time lapse video of iPSC-NPCs growing and expressing GFP over 21 day period: Scan with Layar app.

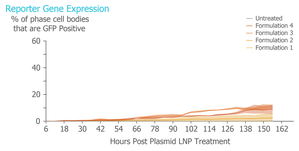
Treatment Details

Cell Type	iPSC-Derived NPCs
N/P Ratio	6
Payload	GFP Plasmid
Treatment Point	Day 1 of differentiation protocol
Dose	0.6 µg DNA /mL media
Apo-E	1 µg/mL media
Biological Assay	Live-cell imaging: Phase: Neurite Length Fluorescence: GFP Expr.
Capture Frequency	6 Hours
Total Observed Period	21 Days

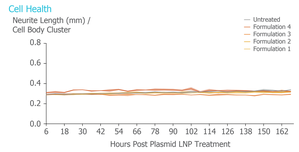
Systematic screening of plasmid LNP formulations in mature human iPSC-derived cortical neurons: N/P ratio had a profound impact

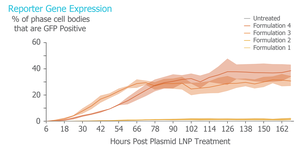
A N/P 6: Impacts Cell Health and Has Low Reporter Expression

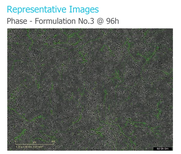


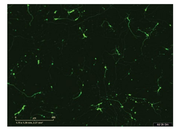


B N/P 4: No Impact on Cell Health and Has Strong Expression of Reporter Gene









- The N/P ratio is important to formulation efficacy for plasmid delivery to human iPSC-derived cortical neurons.
- Different N/P ratios were tested to identify candidate formulations with optimal performance in this therapeutically relevant cell type.

Time lapse video of iPSC-derived cortical neurons growing and expressing GFP over 6 day period: Scan with Layar app.

Treatment Details

Cell Type	iPSC-Derived NPCs
N/P Ratio	4
Payload	GFP Plasmid
Treatment Point	Day 14 of differentiation
Dose	0.6 µg DNA /mL media
Apo-E	1 µg/mL media
Biological Assay	Live-cell imaging: Phase: Neurite Length Fluorescence: GFP Expr.
Capture Frequency	6 Hours
Total Observed Period	7 Days

Conclusions

- Microliter formulations containing microgram quantities of mRNA were rapidly produced using the NanoAssemblr Spark microfluidic system to systematically screen compositions against properties and activity
- PEG-lipid content ≥ 4 mol% reduced encapsulation efficiency; PNI-ILa produced LNPs with more favourable size and PDI than other cationic lipids; N/P ratios > 6 did not appreciably affect properties
- A panel of 4 formulations formulated at N/P 6 and N/P 4 were rapidly developed for testing in iPSC-derived neurons
- On iPSC-derived NPCs, formulations 3 and 4 outperformed 1 and 2 in terms of cell tolerance and reporter gene expression
- On iPSC-derived cortical neurons, all formulations at N/P 4 were better tolerated than N/P 6, and formulations 3 and 4 at N/P = 4 were most effective in inducing reporter gene expression
- Despite similar phsyical properties, formulations had very different in vitro efficacy
- The Spark is ideal for efficient screening of formulations with valuable payloads in clinically relevant cell types