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Unlocking Efficiency in LNP Development: Cutting-Edge Tools for Characterization and *In Vitro* Screening

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Abstract

In the rapidly evolving field of lipid nanoparticle (LNP) research, the integration of advanced technologies is crucial for the efficient generation, screening, and characterization of formulations. This application note highlights a streamlined workflow of innovative techniques used to develop and assess LNP formulations. Utilizing the Sunscreen® high-throughput screening (HTS) equipment alongside the LipidBrick® Library, which features a unique collection of proprietary cationic lipids, researchers can achieve efficient formulation development. The note also details the characterization of LNPs using the new Stunner AF® (Add Fluorescence), which offers high-throughput and accurate analysis of particle size and encapsulation efficiency (EE). Additionally, it covers the *in vitro* screening of LNP functionality using the Incucyte® Live-Cell Imaging System, enabling thorough evaluation of transfection efficiency across various cell lines and immune cells. This approach facilitates the optimization of formulations tailored to specific cell types and primary T cells. We explore how these advanced tools and methodologies are transforming LNP research and formulation development.

UNCHAINED LABS

Introduction

Lipid nanoparticles (LNPs) have emerged as a pivotal technology for the delivery of nucleic acid-based therapeutics, driven by the approval of the first antisense RNA (RNAi) therapeutics (Onpattro®) approved by both the US FDA and EMA in 2018, and more recently the tremendous success of mRNA vaccines against the coronavirus disease 2019 (COVID-19; Pfizer-BioNTech's Comirnaty® and Moderna's Spikevax®).[1,2,3] Despite their potential, the formulation, characterization, and screening of LNPs prior to *in vivo* application present significant challenges. Current technologies often fall short in providing the reliability and efficiency needed to ensure optimal performance, particularly in terms of transfection efficiency, stability, and targeted biodistribution. Besides, a significant limitation of ionizable lipids, the most frequently used active lipids in LNPs, is their tendency to accumulate preferentially in the liver after intravenous (IV) administration, making access to most targets challenging. The use of cationic lipids offers an alternative, enabling targeted delivery to the lungs and spleen.

The demand for reliable and efficient LNPs is coupled with the necessity for rapid characterization methods that can accommodate high-throughput screening. This flexibility is crucial for customizing LNP formulations to achieve specific biodistribution profiles, ensuring that therapeutic agents reach their intended targets effectively. High-throughput characterization and screening are essential to streamline the development process and enhance the precision of LNP applications.

This application note demonstrates the advancements in LNP formulation and characterization, showcasing the use of cutting-edge technologies to overcome existing limitations. By utilizing the Sunscreen® HTS system in combination with the LipidBrick® library, it facilitates efficient LNP formulation development. The characterization of LNPs was conducted using the Stunner AF® (Add Fluorescence), which allows for high-throughput analysis of particle size and EE. Additionally, transfection efficiency was assessed *in vitro* across different cell types using the Incucyte® Live-Cell Analysis System, offering insights into the swift and effective screening of LNPs and underscoring the potential for customized biodistribution and enhanced therapeutic results.

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Materials and Methods

The following methods outline a flexible, streamlined workflow for the development and characterization of LNP formulations (Figure 1).

LNP Formulation

All lipids were dissolved in ethanol at various concentrations: LipidBrick® lipids from Sartorius Polyplus (IM3c, IM12c, IM13c, IM15c, IM16c, IM21.7c, IM22c and IM25c or IMXXc) and SM- 102 (100 mM), DOPE and DSPC (50 mM), Cholesterol (50 mM), DMG-PEG (10 mM), and final molar ratios (%) established as shown in Table 1. eGFP mRNA was diluted in 50 mM sodium citrate (pH 4.0) buffer prior to LNP fabrication. mRNA containing LNPs were prepared by combining the lipid and mRNA solutions in the Sunscreen® system from Unchained Labs at a speed of 10 mL/min and volumetric ratio of 3:1 (Aqueous/Organic), followed by removal of ethanol with dialysis (10 kDa molecular weight cutoff) against PBS 1X at +4°C. Materials used are outlined in Table 2.

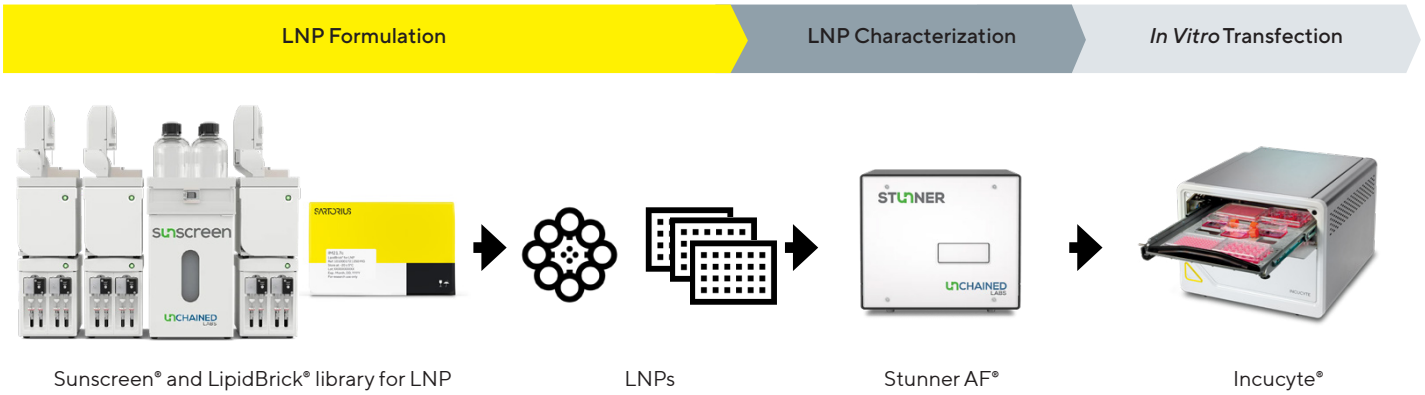

Figure 1. Schematic showcasing the use of Sartorius and Unchained platforms in development and characterization of LNP formulations. Sunscreen® HTS system can be used in combination with the LipidBrick® library for efficient LNP formulation development. LNPs are characterized using the Stunner AF® (Add Fluorescence), which enables high-throughput analysis of particle size and EE. Transfection efficiency can subsequently be screened *in vitro* across various cell types using the Incucyte® Live-Cell Analysis System.

LNP Compositions					
IMXXc 50 %	IMXXc 20 %	IMXXc 20 %	IMXXc 20 %	IMXXc 50 %	IMXXc 40 %
Or	DOPE 40 %	DOPE 30 %	DOPE 50 %	DSPC 10 %	DSPC 20
SM-102 50 %	Chol 38.5 %	Chol 48.5	Chol 28.5	Chol 38.5 %	Chol 38.5 %
DOPE 10 %	PEG 1.5 %	PEG 1.5 %	PEG 1.5 %	PEG 1.5 %	PEG 1.5 %
Chol 38.5 %					
PEG 1.5 %					

Table 1. LNP formulations.

Materials	Supplier	Cat. No.
LipidBrick® Library kit: IM3c, IM12c, IM13c, IM15c, IM16c, IM21.7c, IM22c and IM25c	Sartorius Polyplus	101000241
SM-102	Avanti Polar Lipids, Inc	792885
DOPE	Avanti Polar Lipids, Inc	850725P
DSPC	Avanti Polar Lipids, Inc	850365P
Cholesterol	Sigma-Aldrich	C3045
DMG-PEG	Avanti Polar Lipids, Inc	880151P
Clean Cap® EGFP mRNA (5moU)	TriLink BioTechnologies	L-7201-5
Sunscreen® System	Unchained Labs	7206046

Table 2. Materials used for LNP formulations.

LNP Characterization

LNPs were characterized using Stunner AF® from Unchained Labs, which simultaneously assesses sizing and EE by reading fluorescence, UV/Vis concentration and dynamic light scattering within 2 µL microfluidic wells. EE, total RNA concentration, and free RNA concentrations were calculated using the RNA-LNP EE application and materials used are outlined below (Table 3). Total mRNA concentration in LNPs was measured using UV/vis absorbance and advanced deconvolution algorithms to separate RNA absorbance from other components, like cholesterol and turbidity. EE of LNPs was determined by assessing free mRNA with the fluorescent Quant-iT™ RiboGreen™ RNA Assay Kit. LNPs were diluted in Tris-EDTA Buffer with Ribogreen Reagent diluted 60-fold then free-mRNA was assessed by fluorescence reading (ex/em 485/520nm).

EE was determined using the following equation:

$$EE\ (\%) = \frac{total\ mRNA - free\ mRNA}{total\ mRNA} \times 100$$

For sizing characterization, rotating angle dynamic light scattering (RADLS) was used on Stunner AF to gather static and DLS data from multiple angles in each read. RADLS reads were used for detecting the size and PDI of LNPs and screen for the presence of aggregates.

Materials	Supplier	Cat. No.
Quant-iT™ RiboGreen™ RNA Assay Kit	ThermoFisher Scientific	R11490A
Stunner AF®	Unchained Labs	700-3003

Table 3. Materials used for LNP characterization

Cell Culture

Human embryonic kidney 293 cells (HEK-293), A498 human renal carcinoma cells, HuH7 human hepatocellular carcinoma cells, and RAW 264.7 murine macrophage tumor cells, were grown in flasks and cultured in media as described below (Table 4). Human primary T cells from healthy donors were isolated from peripheral blood by magnetic activated cell sorting and were frozen for later use. Thawed T cells were cultured in complete medium at a density of $1.5x10^6$ cells/ml in a flask and activated with T Cell TransAct™ for 72 hours in a 37 °C and 5% CO_2 humidified incubator.

Media Component	Supplier	Cat. No.	HEK-293 Medium	A498 Medium	HuH7 Medium	RAW264.7 Medium	T-Cell Medium
EMEM	Sigma-Aldrich	M2279-500ML	Base	Base	-	-	-
DMEM	Sigma-Aldrich	D5546-500ML	-	-	Base	Base	-
FBS	Biowest	S1810-500	10%	10%	10%	10%	-
10X Penicil-lin-Streptomycin	Thermo Fisher Scientific	15140-122	2%	2%	2%	2%	1%
100X AANE	Gibco	11140-035	1%	1%	-	-	-
200 mM L-Glutamine	Sigma-Aldrich	G7513-100ML	1%	1%	1%	1%	-
100 mM Na Pyruvate	Sigma-Aldrich	S8636-100	-	1%	-	-	-
CellGenix GMP TCM	Sartorius CellGenix	20814-0500	-	-	-	-	Base
IL-7	Sartorius CellGenix	1410-050	-	-	-	-	10 ng/mL
IL-15	Sartorius CellGenix	1413-050	-	-	-	-	10 ng/mL
T Cell TransAct™	Miltenyi Biotec	130-111-160	-	-	-	-	10 µL/106 cells

Table 4. Media formulations used for cell culture.

In vitro LNP Transfection

For transfection experiments, cells were seeded into 96-well plates in complete medium one day before transfection for adherent cell lines and on the day of transfection for T cells. On the day of transfection, dialyzed or non-dialyzed LNPs were added dropwise to cells in their complete growth medium. Cell and LNP densities used are shown below (Table 5). SM-102 LNP was used as a positive control. For T cell transfection, Apolipoprotein E4 (ApoE4) was added to the cells before adding SM-102 LNP but not for the other LNPs. 4 hours after transfection, 175 µL fresh medium was added with Sartorius Opti-Green background suppressor to remove green autofluorescence from the media. Transfection efficiency was monitored in the Incucyte® Live-Cell Analysis System (Incucyte® System) for up to 48 hours.

High-definition (HD) phase contrast and green fluorescence images were acquired every 3 hours using a 10x objective (4 images/well). Images were analyzed using the integrated Incucyte® Cell-By-Cell Analysis Software module for the cell density, GFP mean intensity, and classification of cells based on green fluorescence (% GFP). The Incucyte® Advanced Label-Free Classification (ALFC) Software module was used to assess cell viability by training the classifier to identify live or dead cells label-free based on 25 different morphology metrics. Materials used are outlined in Table 6.

Cell Type	Cell Density per Well	LNP Amount	LNP Dialyzed
HEK-293	7,500	37.5 ng	No
A498	2,500	50 ng	No
HUH7	7,500	50 ng	No
RAW 264.7	15,000	150 ng	Yes
Human Primary T Cells	187,500	100 ng	Yes

Table 5. Cell and LNP densities used for *in vitro* LNP transfection

Media Component	Supplier	Cat. No.	Final Concentration
Apolipoprotein E4 (ApoE4)	Peprotech	350-04	5 µg/ mL
Incucyte® Opti-Green Reagent (supplied with Incucyte® Mouse IgG1 Fabfluor Antibody Labeling Dye)	Sartorius	4745	0.5 µM
Incucyte® Live-Cell Analysis System	Sartorius	4647	-

Table 6. Materials used for *in vitro* LNP transfection

Results

LNP Formulation

Screening various LNP compositions is crucial when aiming to identify a specific formulation from a lipid library, as it allows for the evaluation of diverse lipid interactions and their effects on the physical characteristic particles. These include the size, the zeta potential, or the EE, and on the transfection efficiency, which can vary depending on the cell type and/or tissue of interest. This comprehensive approach ensures the selection of the most effective LNP composition tailored to specific research or therapeutic needs. Here, eight different cationic lipids were used from the LipidBrick® library: IM3c, IM12c, IM13c, IM15c, IM16c, IM21.7c, IM22c and IM25c (Table 2). For LNP formulations, two types of phospholipids were used (DSPC and DOPE). Various proportions of cationic lipid (20-50%), phospholipid (10-50%), and cholesterol (28.5-48.5%) were screened, while maintaining a fixed percentage of DMG-PEG lipid at 1.5%.

The Sunscreen® system uses fluid handling robotics and microfluidic mixing to automate LNP preparation, using 96 well plates for reagent input and sample collection. Utilizing the Sunscreen HTS equipment, 56 LNPs were efficiently generated in under 4 hours using a single 96-well plate, compared to the minimum of 6 hours required if each LNP were produced individually.

Rapid LNP Characterization

Stunner AF® simultaneously characterizes LNPs based on sizing and EE by reading fluorescence, UV/Vis concentration and DLS in microfluidic wells. The utilization of the Stunner AF® significantly enhances the efficiency of LNP characterization, allowing for rapid analysis. 56 LNPs in both dialyzed and non-dialyzed states were characterized within just 20 hours with only 3 hours of direct involvement, while the remaining 17 hours were spent on automated running analysis. This automated approach contrasts sharply with the manual process, which would require over 30 hours to complete both size and EE analysis which represents more than three full working days with someone constantly at the bench. The ability of the Stunner AF® to streamline the characterization process not only saves valuable time but also ensures consistent and accurate results, making it an indispensable tool for high-throughput LNP analysis.
DLS analysis reveals that all LNP formulations are well-suited for in vivo applications, with particle sizes consistently measuring below 200 nm, ranging from 87 to 172 nm for dialyzed LNP formulations (Figure 2A) and from 94 to 193 nm for non-dialyzed LNP formulations (data not shown). This optimal size range is crucial for improved tissue penetration, effective cellular uptake, and reduced risk of aggregation, ensuring efficient biodistribution in vivo applications. Furthermore, the EE of all LNPs (dialyzed and non-dialyzed) exceeds 80 %, with nearly all dialyzed LipidBrick® LNP formulations (49 out of 53) achieving similar or greater efficiency compared to the control, SM-102 (Figure 2B).

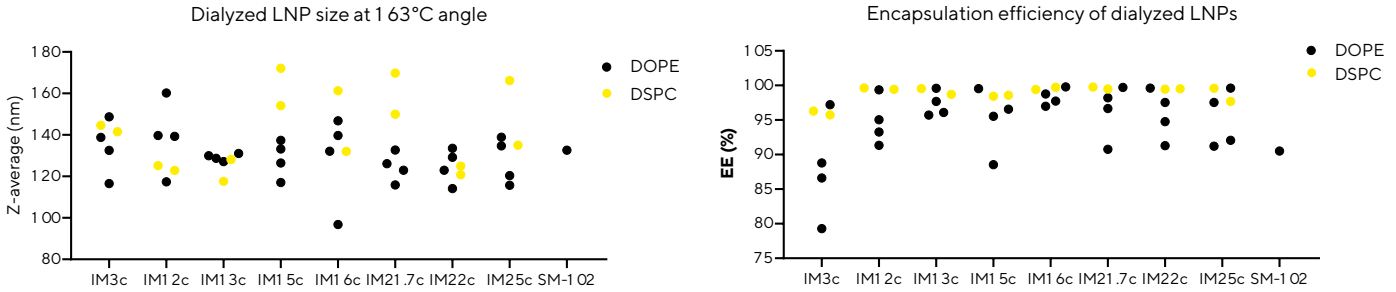

Figure 2. Quantification of LNP size and EE using Stunner AF®. 56 LNPs were characterized using Stunner AF. A) Dot plot of Z-average of dialyzed LNPs. B) Dot plot of EE percentage of dialyzed LNPs. Data presented as mean + SEM, n = 3 replicates and each dot represents one LNP composition.

In vitro LNP Screening Using Live-Cell Analysis

Live-cell analysis enables the visualization and quantification of fluorescent LNPs *in vitro*. This real-time monitoring facilitates the tracking of LNP uptake, mRNA delivery, transfection efficiency, and functional expression in live cells whilst simultaneously being able to assess effects on cell growth or viability. This approach allows for rapid, dynamic high-throughput screening of LNP formulations across a range of cell types and is critical for predicting *in vivo* performance.

In this study, HEK-293 cells, which are frequently used in LNP research, were initially employed as a Proof of Concept (POC) to demonstrate that live-cell analysis can facilitate the identification of effective LNP formulations, prior to transitioning to other cell types of greater interest. HEK-293 cells were treated with 56 LNP formulations and monitored using the Incucyte® System over 48 hours (Figure 3). Representative images are shown and illustrate successful *in vitro* transfection as indicated by an increase in green (GFP) fluorescence (Figure 3A). Using integrated image analysis, individual cells were segmented and then classified into high or low GFP expressing populations (teal or magenta outlines, respectively; Figure 3B), offering insights into the transfection efficiency. The non-transfected (NT) cells serve as a baseline, illustrating the natural state of the cells without any LNP transfection. As anticipated, the control LNP with the ionizable lipid SM-102 shows some cells expressing GFP after 48 hours of transfection. In contrast, the two LNP formulations containing IM16c or IM13c lipids from LipidBrick® library demonstrate remarkable efficacy, with almost all cells expressing GFP following transfection. Additionally, this analysis allows for the label-free assessment of the effects on cell proliferation and viability, measured via cell density and analysis using ALFC, where a classifier was trained to identify live and dead cells based on morphology (green or red outlines, respectively, Figure 3C). These findings underscore the potential of live-cell analysis in optimizing LNP formulations for enhanced transfection outcomes.

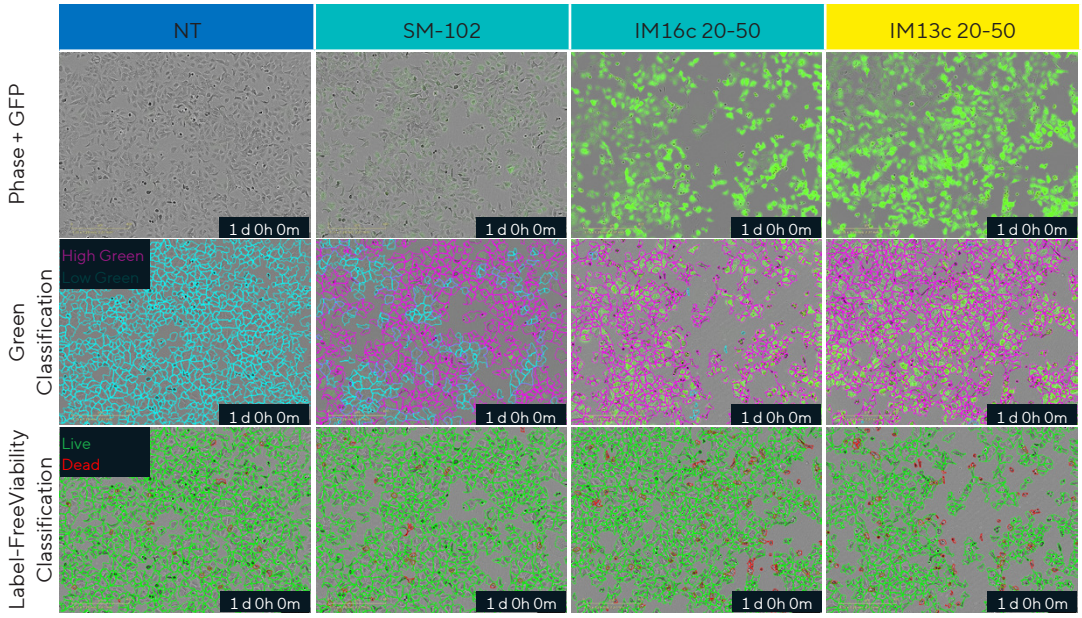

Figure 3. Screening of LNPs in HEK-293 cells utilizing live-cell analysis. HEK-293 cells were treated with LNPs and monitored over 48 hours in the Incucyte® System. A) Representative phase and green fluorescence images shown for non-treated (NT), control LNPs (SM-102) or LipidBrick® LNPs (IM16c, IM13c) at 24 hours. B) Cell-by-Cell classification masks (high green = teal outline, low green = magenta outline) and C) ALFC viability masks (live = green outline, dead = red outline).

When examining the percentage of HEK-293 cells expressing GFP with each LNP formulation, 21 compositions are identified that surpass the control SM-102 LNP formulation. Notably, at least one LNP composition for each lipid from the LipidBrick® library demonstrated superior performance (Figure 4A). Additionally, 38 compositions are identified that meet or exceed the mean intensity of SM-102 LNP formulation (Figure 4B). After an initial high-level overview, attention was directed towards the six top LNP compositions, which exhibited the highest percentage of GFP-expressing cells and the greatest mean intensity. This allowed for a more detailed examination of the kinetic analysis of LNP transfection in HEK-293 cells. Notably, the lipids IM12c, IM13c, and IM16c demonstrate superior performance, with over 90 % of cells transfected within 10 hours compared to SM-102 where only 70 % of cells are transfected after 24 hours (Figure 4C). Out of these 6 top LNP formulations, 3 are with 20 % of IM13c lipids with the following compositions: IM13c-DOPE-Cholesterol-DMG-PEG ratios of 20 %-40 %-38.5 %-1.5 %, of 20 %-30 %-48.5 %-1.5 %, and 20 %-50 %-28.5 %-1.5. IM12c, IM13c, and IM16c-based LNPs achieve rapid transfection of HEK-293 cells, resulting in higher GFP expression percentage and intensity than SM-102 LNP formulation without impacting cell proliferation and viability (Figure 4D, 4E and 4F).

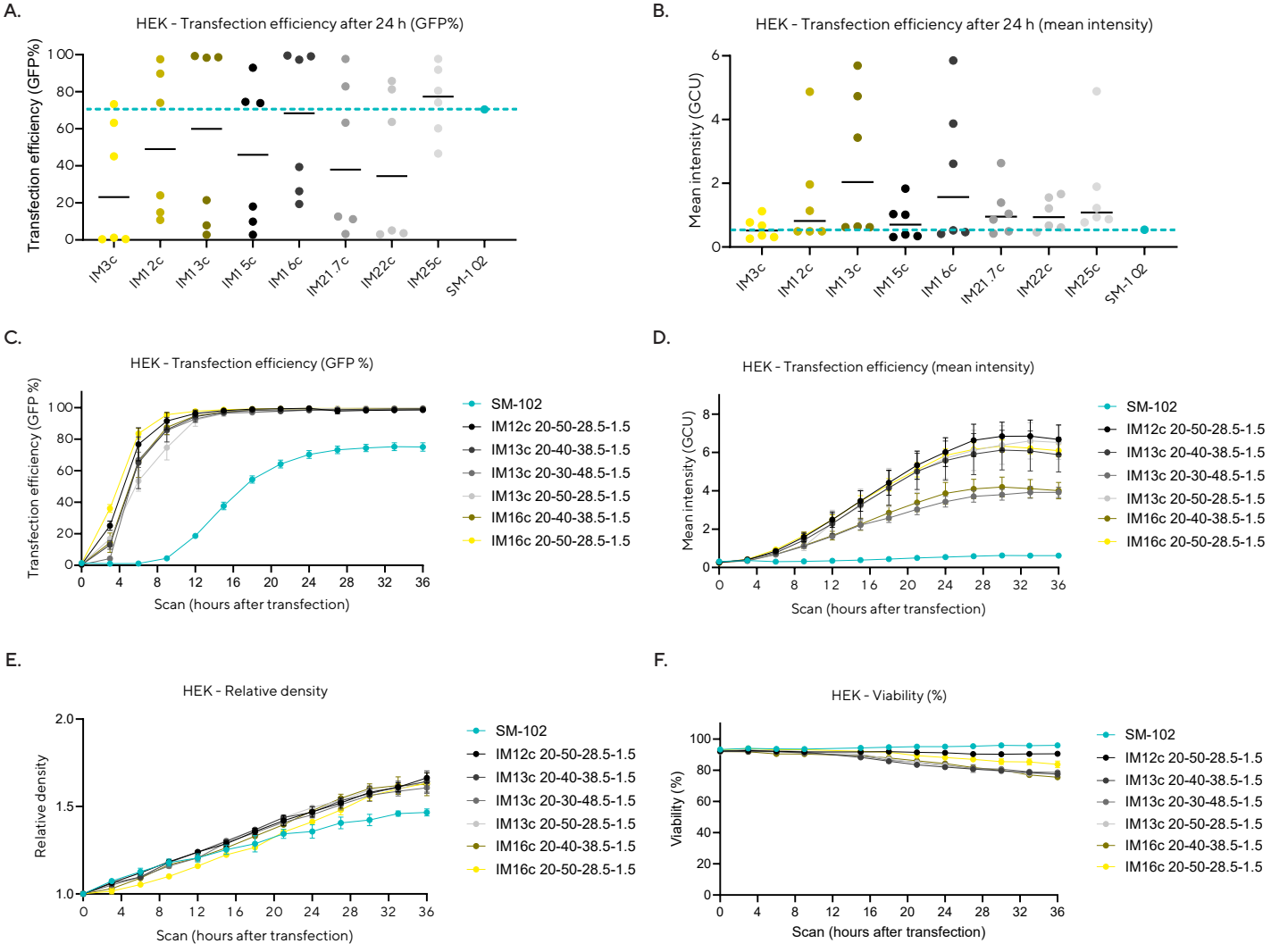

Figure 4. Kinetic analysis of LNPs in HEK-293 cells using live-cell analysis. HEK-293 cells were treated with LNPs and monitored over 48 hours in the Incucyte® System. A) Transfection efficiency (GFP %) shown for all LNPs at 24 hours, where each dot represents one LNP composition. B) Transfection efficiency (GFP Mean Intensity) shown for all LNPs at 24 hours, where each dot represents one LNP composition. C) Time course of transfection efficiency (GFP %) for 6 LNPs compared to SM-102 control. D) Time course of transfection efficiency (GFP Mean Intensity) for 6 LNPs compared to SM-102 control. E) Time course of relative density normalized to t=0 for 6 LNPs compared to SM-102 control. F) Time course of viability for 6 LNPs compared to SM-102 control. Data presented as mean ± SEM.

In Vitro LNP Screening Across Different Cell Types to Identify Cell Specific Formulations for *In Vivo* Applications

The use of the IM21.7c lipid from the LipidBrick® library in LNPs has been previously shown to allow for extra-hepatic biodistribution with a high expression in the lung following IV injection compared to the use of ionizable lipids in LNPs where the expression will stay in the liver.[4] To assess the efficacy of additional lipids from the LipidBrick® library, two cell lines were selected: A498 cells, a cell line with epithelial morphology isolated from kidney tissue, and HuH-7 cells, a type of epithelial-like tumorigenic cell line isolated from a liver tumor. The aim was to identify which LNPs would result in selective organ targeting (SORT) nanoparticles for the lung (A498 cells) or liver (HuH-7cells).[5]

For A498 cells, after 48 hours of transfection, 5 LNP compositions containing lipids from the LipidBrick® library show a similar percentage of GFP-expressing cells compared to the control SM-102-based LNP (Figure 5A) and 15 LNPs compositions are also identified that meet or exceed SM-102 in mean intensity (Figure 5B). The top-performing lipids, IM13c, IM15c and IM16c, demonstrate strong transfection efficiency in terms of GFP expression percentage and intensity. However, variations in cell viability are noted, with IM16c having a more pronounced impact on viability.

In HuH-7 liver cells, consistent with previous *in vivo* observations, LNPs formulated with cationic lipids are less effective at transfecting hepatocytes. All tested LNPs exhibited lower GFP expression percentages compared to the control LNP formulated with the SM-102 ionizable lipid. However, a few LNP compositions with cationic lipids surpassed the SM-102 LNP composition in mean intensity, with those based on IM12c and IM21.7c predominantly standing out, without impacting cell viability.
These findings underscore the potential of the LNP formulations with IM13c, IM15c and IM16c lipids for successful *in vivo* lung delivery.

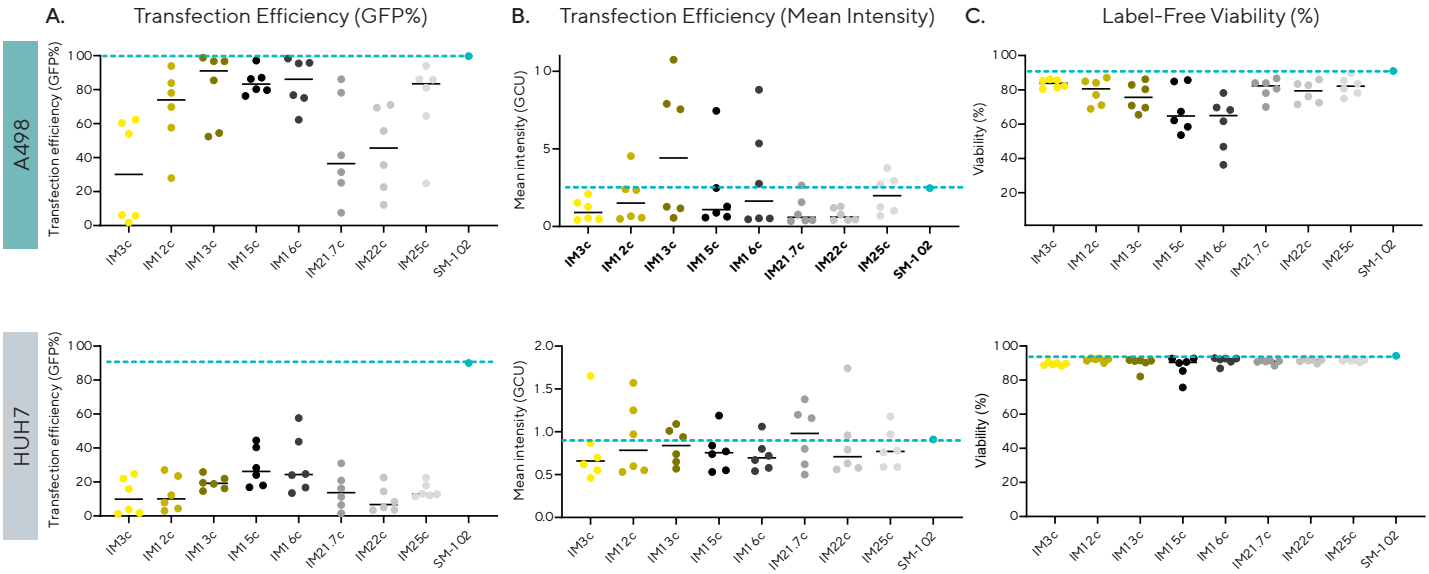

Figure 5. LNP *in vitro* screening in A498 and HUH7 cells using live-cell analysis. A498 and HuH7 cells were treated with LNPs and monitored over 48 or 24 hours, respectively in the Incucyte® System. A) Transfection efficiency (GFP %) shown for all LNPs at 48 hours for A498 cells and at 24 hours for HuH7 cells. B) Transfection efficiency (GFP Mean Intensity) shown for all LNPs at 48 hours for A498 cells and at 24 hours for HuH7 cells. C) Label-free viability shown for all LNPs at 24 hours. Data presented as mean ± SEM, where each dot represents one LNP composition. masks (live = green outline, dead = red outline).

In Vitro LNP Screening of Immune Cells

For the *in vitro* screening of immune cells, we utilized RAW 264.7 macrophages and human primary T cells to evaluate the efficacy of LNP formulations.

For RAW 264.7 cells, when analyzing the percentage of cells expressing GFP with different LNP formulations, 12 compositions are identified that match or exceed the control SM-102-based LNP. Notably, nearly all LNP compositions surpass SM-102 in mean intensity as observed in the representative phase and fluorescence images for lipids IM21.7c and IM25c (Figure 6A). The lipids IM16c, IM21.7c, and IM25c demonstrate superior performance, with up to 70 % of cells transfected within 18-24 hours compared to SM-102 where only 53 % of cells are transfected after 39 hours (Figure 6B). IM16c, IM21.7c, and IM25c achieve rapid transfection of RAW 264.7 cells, resulting in higher GFP intensity than SM-102 without impacting the cell the viability (Figure 6C and 6D).

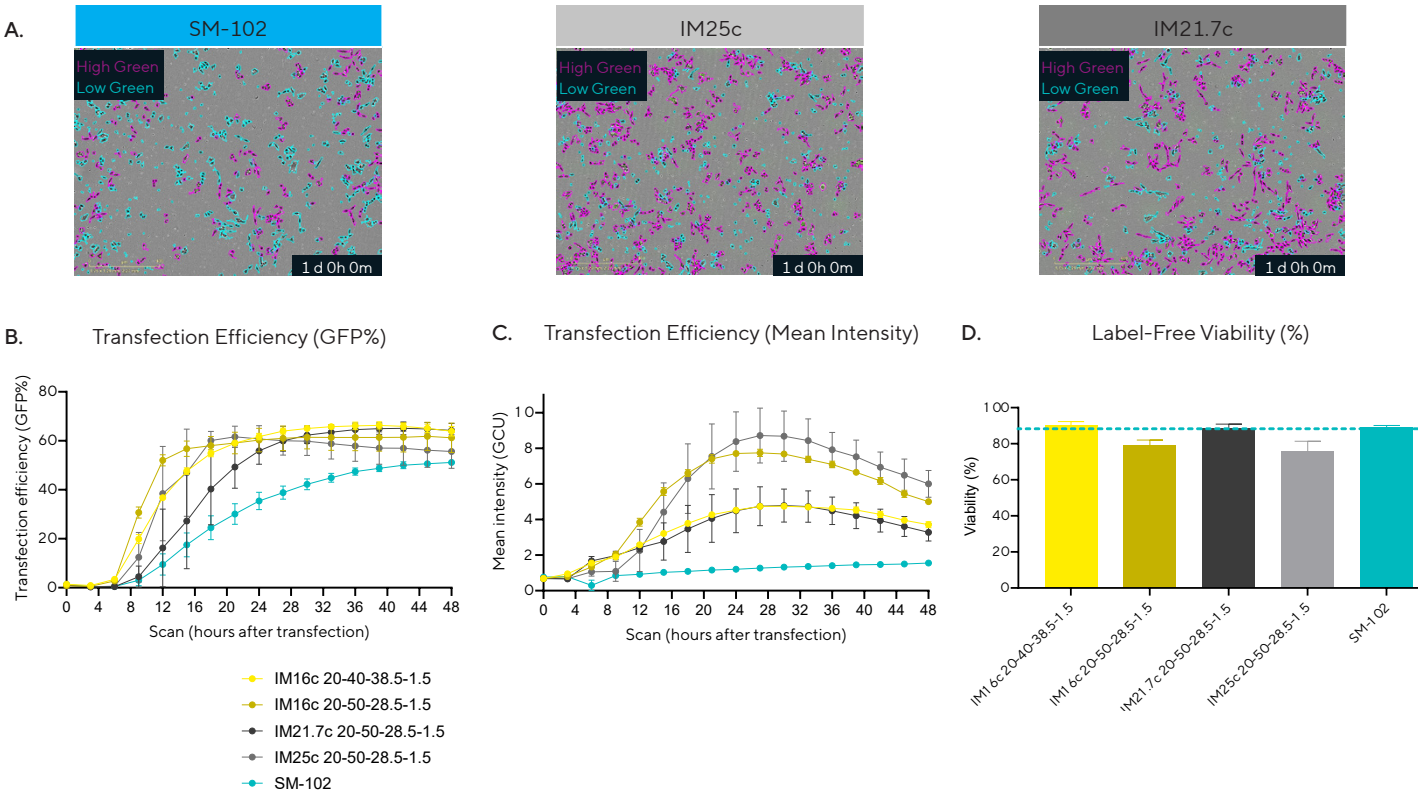

Figure 6. LNP *in vitro* screening in immune cells using live-cell analysis. RAW 264.7 cells were treated with LNPs and monitored over 48 hours in the Incucyte® System. A) Representative phase and fluorescence images at 24 hours. Outlines shown for Cell-by-Cell classification masks (high green = teal outline, low green = magenta outline). B) Time course of transfection efficiency (GFP %) for 4 LNPs compared to SM-102 control. C) Time course of transfection efficiency (GFP Mean Intensity) for 4 LNPs compared to SM-102 control. D) Viability for 4 LNPs compared to SM-102 control at 24 hours. Data presented as mean ± SEM, n = 3 replicates.

In primary T cells, when examining the percentage of T cells expressing GFP with various LNP formulations, 16 compositions are found to match or exceed the control SM-102 LNP, with at least one LNP composition for each lipid from the LipidBrick® library, except for IM3c. Additionally, 39 compositions are identified that surpass SM-102 in mean intensity. The lipids IM16c, IM21.7c, and IM22c exhibited superior performance, transfecting up to 52 % of cells within 12 hours, compared to SM-102, which reached peak expression of 25 % after 18 hours (Figure 7A).

Notably, LNP formulations with cationic lipids did not require the addition of ApoE4 for effective mRNA delivery, unlike SM-102, which relies on ApoE4 to bind T cells through LDL receptors.[6] Furthermore, with IM16c, IM21.7c, and IM25c lipids, nearly all cells were transfected within just 3 hours, whereas GFP expression in SM-102 LNPs began only after 6 hours. In conclusion, IM16c, IM21.7c, and IM25c achieved rapid transfection of T cells, resulting in higher GFP intensity than SM-102, without affecting cell proliferation (Figure 7B and 7C).

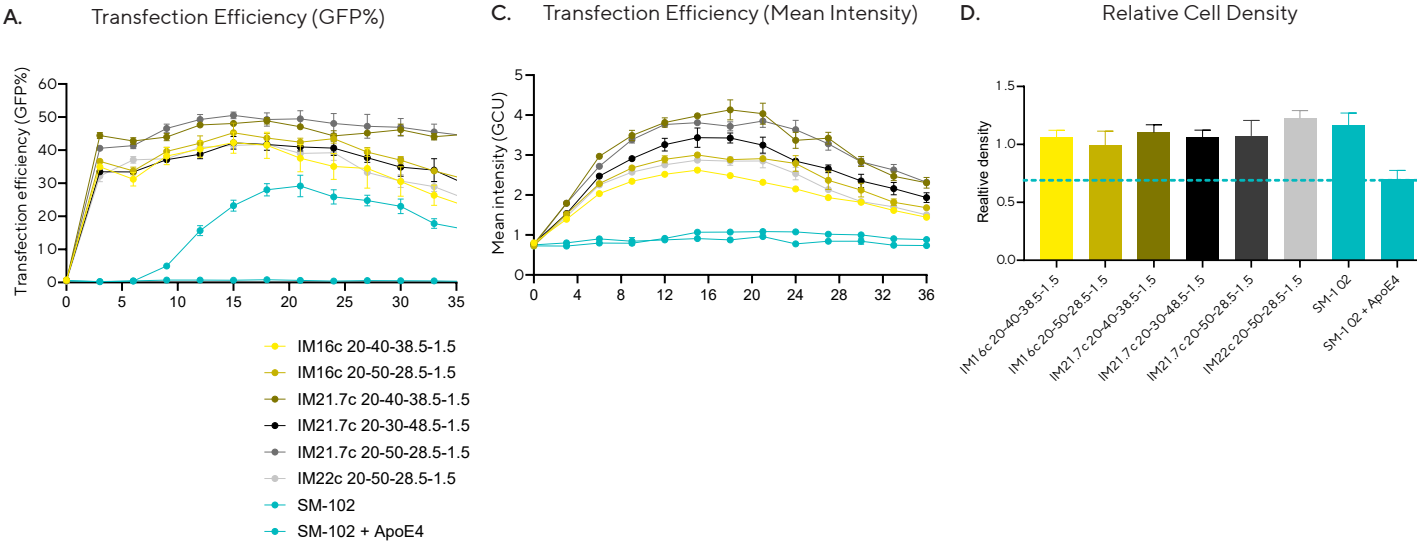

Figure 7. LNP *in vitro* screening in primary immune cells using live-cell analysis.. Human T-cells were treated with LNPs and monitored using live-cell analysis. A) Time courses of transfection efficiency (GFP % or GFP Mean Intensity) shown for 5 LNPs. B) Transfection efficiency (GFP % or GFP Mean Intensity) shown for LNPs at 24 hours. C) Relative density normalized to t=0 shown for LNPs at 24 hours. Data presented as mean ± SEM, n = 3 replicates.

Summary and Outlook

This study highlights the importance of screening diverse LNP compositions to pinpoint optimal formulations from the LipidBrick® library, focusing on transfection efficiency, cell specificity, and cell viability. Utilizing advanced equipment like Sunscreen® HTS and Stunner AF®, we efficiently generated and characterized 56 LNPs, demonstrating their suitability for *in vivo* applications with particle sizes under 200 nm and EE exceeding 80 %. Live-cell analysis enabled us to comprehensively screen different LNP formulations across various cell lines and immune cells and facilitated the optimization of specific formulations tailored to particular cell types. The results revealed superior transfection performance of LipidBrick® lipids, particularly IM12c, IM13c, and IM16c, in HEK-293 cells, achieving rapid and high GFP expression without impacting cell proliferation. Further evaluations on A498 cells identified IM13c, IM15c, and IM16c as promising candidates for lung delivery. The study underscores the potential of these formulations for therapeutic use, paving the way for targeted organ delivery and efficient mRNA transfection in immune cells, including RAW 264.7 macrophages and human primary T cells. Additionally, it opens up possibilities for immune cell delivery *ex vivo* and *in vivo* applications, such as CAR T cell therapies.

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