Simplifying Progress

Integrating Live-Cell Analysis for targeted *In Vitro* LNP Screening

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Introduction

- In the rapidly evolving field of lipid nanoparticle (LNP) research, integrating advanced technologies is essential for efficiently generating, screening, and characterizing formulations.
- This study details the *in vitro* screening of LNP functionality using the Incucyte® Live-Cell Analysis System, enabling a thorough evaluation of transfection efficiency across various cell lines and immune cells.
- This approach facilitates the optimization of formulations tailored to specific cell types, including primary T cells.

Proof-Of-Concept in HEK-293 Cells

- To demonstrate the utility of Live-cell analysis for the visualization and quantification of fluorescent LNPs *in vitro*, HEK-293 Cells were treated in 96-well plates with 56 LNP formulations and monitored using the Incucyte® System over 36 hours.
- Representative images show successful transfection as indicated by an increase in green (GFP) fluorescence (Fig 1 A). Using Incucyte® Cell-by-Cell Analysis Software, individual cells were segmented and classified into high or low GFP expressing populations (Fig 1B), offering insights into the transfection efficiency.
- The non transfected (NT, first column) cells serve as a baseline.
- Control LNP, SM- 102 (characterized ionizable lipid) shows some cells expressing GFP.
- Two LNP formulations demonstrated remarkable efficacy, with almost all cells expressing GFP following transfection.
- A label-free assessment was used to investigate effects on cell proliferation (cell density) and viability. Utilizing Incucyte® Advanced Label-Free Classification (ALFC), a classifier was trained to identify live and dead cells based on morphology (Fig 1C).
- These findings underscore the potential of live-cell analysis in optimizing LNP formulations for enhanced transfection outcomes.

Details of LNP compositions used in this work can be found in the following application note - Unlocking Efficiency in LNP Development: Cutting-Edge Tools for Characterization and *In Vitro* Screening available at Sartorius.com or by following in the QR code above.

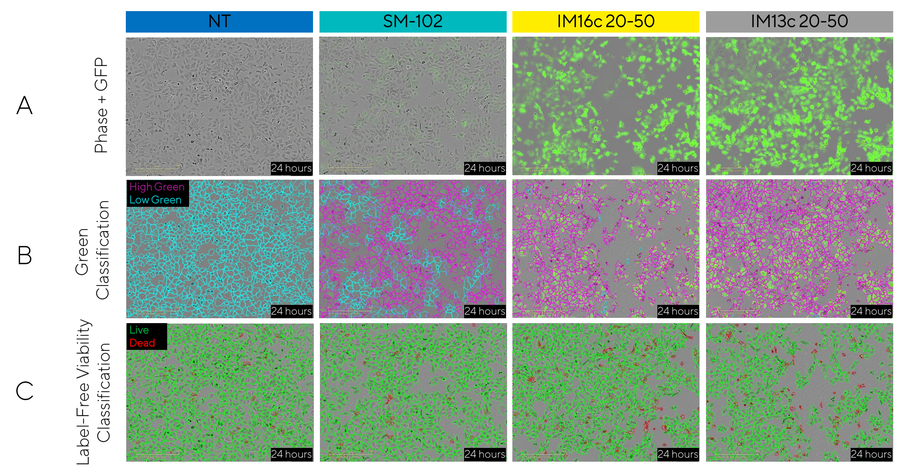


Figure 1: Screening of LNPs in HEK-293 cells. A) Representative phase and green fluorescence images at 24 hours. B) Cell-by-Cell classification masks (high green = teal outline, low green = magenta outline) and C) ALFC viability masks (live = green outline, dead = red outline).

- Multiple compositions displayed greater GFP expression (either GFP% or mean intensity) than the control SM-102 LNP formulation, dotted teal line (Fig 2A).
- The six top performing LNP compositions, which exhibited the highest percentage of GFP-expressing cells and the greatest mean intensity were analyzed in greater detail for kinetic responses.
- IM12c, IM13c, and IM16c demonstrate superior performance, achieving rapid transfection of HEK-293 cells with over 90 % of cells transfected within 10 hours compared to SM-102, 70 % of cells are transfected after 24 hours (Fig 2B).
- Transfection was achieved with minimal impacting cell proliferation and viability (Fig 2C).

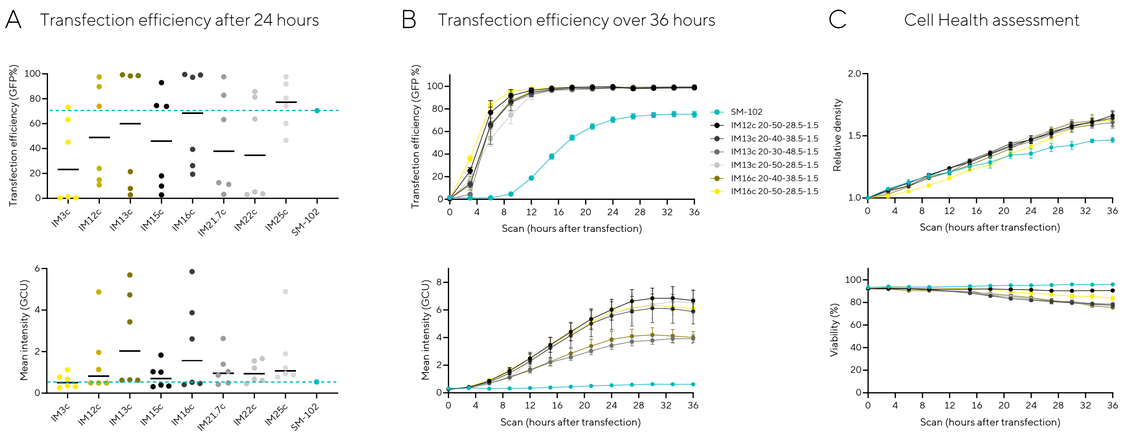


Figure 2: HEK-293 cells were treated with LNPs and monitored over 36 hours. A) Transfection efficiency (GFP % or GFP Mean Intensity) shown for all LNPs at 24 hours, where each dot represents one LNP composition. B) Time course of transfection efficiency (GFP % or GFP Mean Intensity) for 6 LNPs. C) Time course of cell health assessment relative density normalized to t=0 or viability for 6 LNPs. Data presented as mean ± SEM.

Selective Organ-Targeting (SORT)

- SORT has previously been demonstrated with some LNP compositions.[1] To assess this, an epithelial A498 cell line isolated from kidney tissue, and epithelial-like tumorigenic HuH-7 cell line isolated from a liver tumor were tested.
- Cells were transfected and assed in the Incucyte® System for GFP expression and viability.
- A498 cells, post 48 hours transfection (top row):
  - 5 LNP compositions show a similar % of GFP expression compared to the control SM-102-based LNP (Fig 3A) and 15 LNPs compositions were identified that were ≥ SM-102 mean intensity (Fig 3B).
  - IM13c, IM15c and IM16c, demonstrate strong transfection efficiency, however, variations in cell viability were seen (Fig 3C).
- HuH-7 liver cells, post 24 hours transfection (bottom row):
  - All tested LNPs exhibited lower GFP % expression compared to the control LNP.
  - A few LNP compositions surpassed the control in terms of mean intensity, with those based on IM12c and IM21.7c predominantly standing out, without impacting cell viability.
- These findings underscore the potential use of these types of live-cell assessments to investigate SORT.

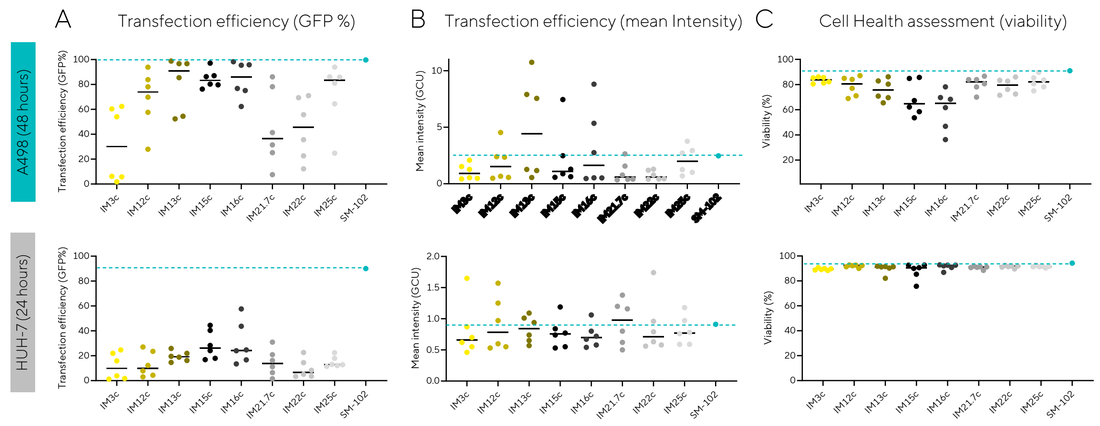
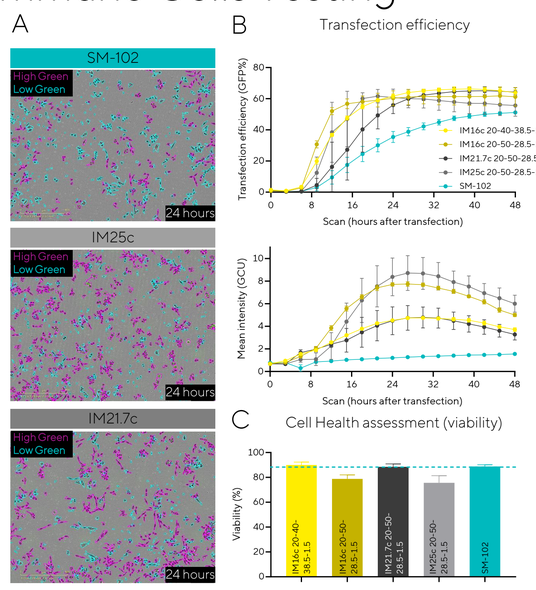


Figure 3. A498 and HuH7 cells A & B) Transfection efficiency (GFP % or mean intensity) shown for all LNPs. C) Label-free viability shown for all LNPs. Data presented as mean ± SEM, where each dot represents one LNP composition.

Immune Cells Testing



To test LNP screening of immune cells, murine RAW 264.7 macrophages and human primary T cells were evaluated.

- RAW 264.7 cells:
  - Multiple LNPs were identified that ≥ GFP% compared to control LNP. With nearly all compositions surpassing mean intensity as shown in the images for lipids IM21.7c and IM25c (Fig 4A).
  - The lipids IM16c, IM21.7c, and IM25c demonstrate rapid transfection, with greater than 60% of cells transfected within 18-24 hours (Fig 4B).
  - No impact on the cell the viability was seen (Fig 4C).

Figure 4. RAW 264.7 cells 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 % or mean intensity) for 4 LNPs. D) Viability for 4 LNPs at 24 hours. Data presented as mean ± SEM, n = 3 replicates.

- Primary T cells: Multiple LNPs ≥ GFP% compared to the control, with addition compositions identified that surpassed SM-102 in mean intensity.
  - Lipids IM16c, IM21.7c, and IM22c transfected up to 52 % of cells within 12 hours (Fig 5A). Notably, some LNP formulations did not require ApoE4 for effective mRNA delivery, unlike SM-102, which relies on ApoE4 to bind T cells.[2]
  - With IM16c, IM21.7c, and IM25c lipids, nearly all cells were transfected within just 3 hours, resulting in higher GFP intensity than control, without affecting cell proliferation (Fig 5B & C).

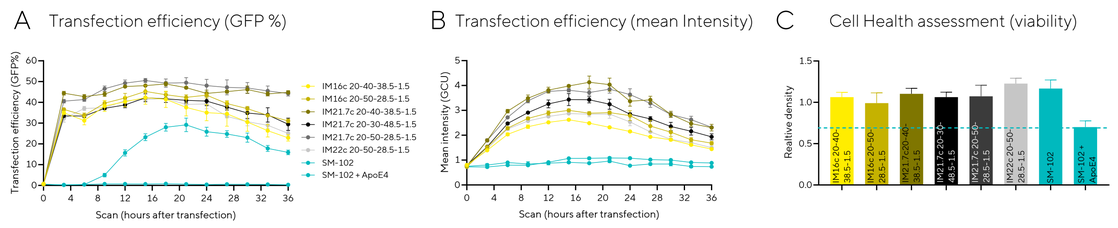


Figure 5. T-cells treated A & B) Time courses of transfection efficiency (GFP % or GFP Mean Intensity) shown for 6 LNPs. C) Relative density normalized to t=0 shown for LNPs at 24 hours. Data presented as mean ± SEM, n = 3 replicates.

Conclusion

This study highlights the utility of live-cell analysis in the rapid screening and optimization of LNP formulations, offering insights into transfection efficiency and cell viability across diverse cell types. When used as part of a broader workflow, it provides a simplified approach that supports *in vitro* insights.

References
1. Guéguen, C. et al., Eur. J. of Pharmaceutics and Biopharmaceutics 195, 114077 (2024).
2. R. Chu, Y. Wang, J. Kong, T. Pan, Y. Yang, J. He. J. Mater. Chem. B, 12 (2024), pp. 4759-4784