Lipid nanoparticle screening in 2D *vs* 3D cultures: Ovarian cancer cell lines exhibit different preference for optimal lipid formulation in monolayers *vs* spheroids

David Taylor, Valerie Forsyth, Caleb Riddering, and Aileen R. Ariosa

Department of Assay Research and Development, Biochemistry Division, Cayman Chemical Company, Ann Arbor, MI

Abstract No.
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KEY FINDING

Optimal LNP formulation for cargo expression differed in 50% of tested conditions when comparing monolayers *vs* spheroids

INTRODUCTION

Cell-based screening is a widely-used fundamental tool in the development of cancer therapeutics. Two-dimensional (2D) culture models often fail, however, to effectively model the complex milieu that comprises the tumor microenvironment.[1] Therapies based on lipid nanoparticles (LNPs) hold potential for tumor therapies where delivery of mRNA cargo can promote antigen expression, enhancing tumor immunity.[2] Thus, there is a need for convenient high-throughput cell-based methods for screening LNP formulations. However, it is unclear how well LNP transfection performance in 2D culture reflects uptake and expression in a three-dimensional (3D) context. Loadable LNPs are empty, preformed LNPs that can encapsulate nucleic acid cargo, enabling testing multiple cargo types in smaller volumes with potential for screening in early formulation development.

In this study, we investigated the expression of three different nucleic acid cargo types (mCherry mRNA, GFP DNA and Cy5-labeled 2′3′-cGAMP) delivered to three different ovarian cancer cell lines *via* four loadable LNPs containing benchmark lipids SM-102, ALC-0315, (S)-C12-200, or DLin-MC3. We then compared the relative efficacy of each formulation in delivering each payload to the various ovarian cancer cells (PA-1, SK-OV-3, and Caov-3) cultured as monolayers *vs* spheroids to assess whether the optimal LNP formulation is consistent across 2D and 3D culture models.

MATERIALS & METHODS

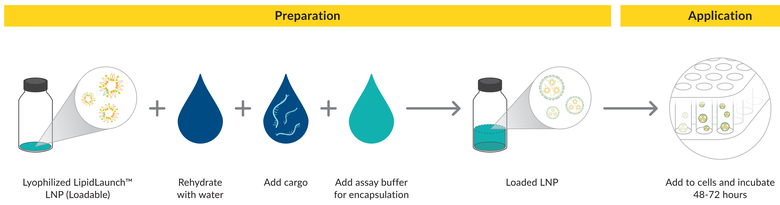

Figure 1 – Overview of cargo loading and transfection via Loadable LNPs.

Monolayer cultures of PA-1, Caov-3, and SK-OV-3 cells were seeded in 96-well plates (4,000 cells/well) 24 h before transfection. mCherry mRNA, GFP plasmid DNA, or Cy5-labeled 2′3′-cGAMP were encapsulated into loadable SM-102, ALC-0315, (S)-C12-200, or Dlin-MC3 LNPs at 6:1 LNP-to-cargo concentrations. Cells were transfected for 48-72 h prior to fluorescence imaging.

Spheroids were seeded in 96-well spheroid culture plates (20,000 cells/well) for 72 h prior to transfections. Spheroids were subjected to the same transfection conditions as monolayer cultures and imaged after 48-72 h. Formulations were then ranked and compared based on transfection efficiency and overall fluorescence intensity.

SUMMARY OF RESULTS

	mCherry mRNA		GFP pDNA		Cy5 2′3′-CGAMP	
	Monolayer	Spheroid	Monolayer	Spheroid	Monolayer	Spheroid
SK-OV-3	(S)-C12-200 ■	(S)-C12-200 ■	(S)-C12-200 ■	SM-102 ■	SM-102 ■	(S)-C12-200 ■
PA-1	(S)-C12-200 ■	(S)-C12-200 ■	ALC-0315 ■	(S)-C12-200 ■	SM-102 ■	SM-102 ■
Caov-3	SM-102 ■	N/A	SM-102 ■	N/A	SM-102 ■	N/A

Figure 2 – Summary of best-performing formulations.

RESULTS

Loadable LNPs exhibited similar average particle sizes determined by dynamic light scattering (DLS, Figure 3). In cell monolayer transfections, SM-102 and (S)-C12-200 LNPs demonstrated the best performance at mRNA transfection in all three tested cell lines (Figure 4). For pDNA and cyclic dinucleotide cargos, results varied by cell type.

Lipid Nanoparticle	Z avg (nm)
SM-102	115 ± 5
ALC-0315	86 ± 4
(S)-C12-200	106 ± 10
DLin-MC3	106 ± 4

Figure 3 – LNP size characterization determined *via* DLS.

Monolayer Model

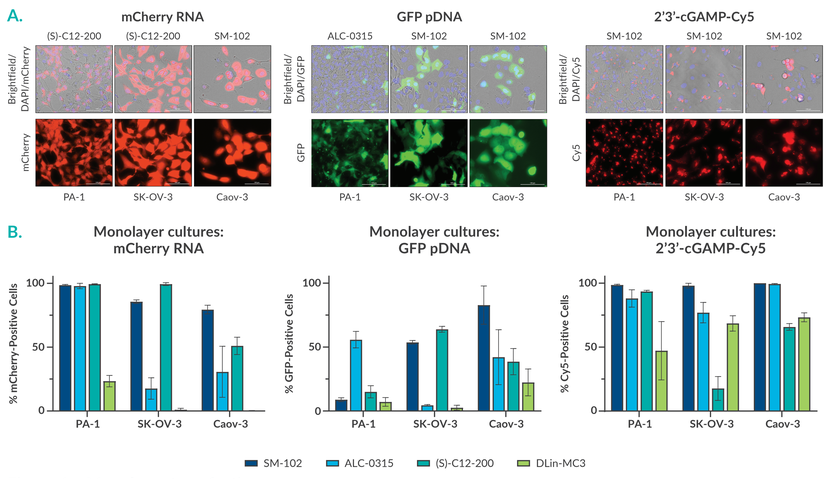

Figure 4 – Monolayer transfection summary.
(A) Brightfield-DAPI-merged and single channel fluorescent images are shown for the best-performing formulation for each cargo and cell type; Bar size: 100 µm. **(B)** Relative transfection efficiency is shown for best-performing condition from each LNP formulation, n=3 wells per condition.

Spheroid Model

Spheroids prepared from each cell line were imaged at Day 3 (Figure 5). PA-1 and SK-OV-3 cells formed coherent masses and exhibited peripheral calcein AM staining patterns which reflected decreasing cell viability towards the core. Caov-3 cells formed loosely aggregated masses with a consistent calcein AM staining throughout. Caov-3 cultures were unable to demonstrate evidence of physicochemical gradients characteristic of spheroids, thus, the spheroid transfection experiments were conducted in PA-1 and SK-OV-3 cells only.

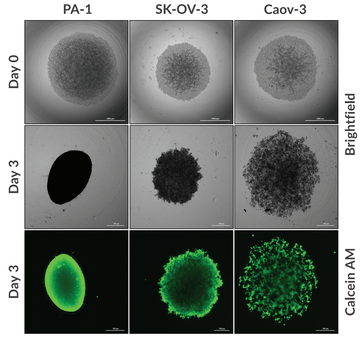

Figure 5 – Spheroid model characterization.
Day 0 and Day 3 brightfield and Day 3 Calcein AM imaging examples are shown for each cell type.

RESULTS (CONTINUED)

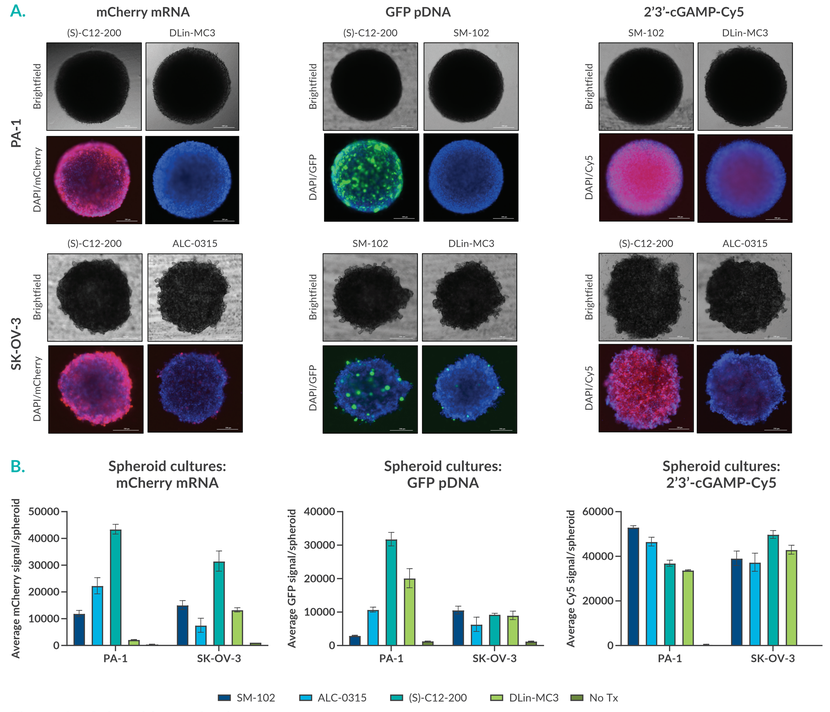

Figure 6 – Spheroid transfection summary.
(A) Brightfield and DAPI-merged fluorescence images are shown for the best- and worst-performing formulation for each cargo and cell type; Bar size: 200 µm. **(B)** Relative fluorescence intensity per spheroid is shown for best-performing condition from each LNP formulation, n=3 wells per condition.

CONCLUSIONS

- Ovarian cancer cell monolayers and spheroids generally exhibited optimal cargo expression in the presence of SM-102 and (S)-C12-200 LNPs.
- Best-performing lipid formulation differed in 50% of tested conditions when comparing monolayer *vs* spheroid transfection results.

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References

1. Nayak, P., Bentivoglio, V., Varani, M., *et al.* Three-dimensional *in vitro* tumor spheroid models for evaluation of anticancer therapy: Recent updates. *Cancers* **15(9)**, 4846, 2023.
2. Kon, E., Ad-El, N., Hazan-Halevy, I. *et al.* Targeting cancer with mRNA-lipid nanoparticles: Key considerations and future prospects. *Nat. Rev. Clin. Oncol.* **20**, 739-754 (2023).