Enhancing mRNA-Lipid Nanoparticle Efficacy

Exploring the Role of Ionisable and Cationic Lipid Combinations on Delivery Efficiency and Immunogenicity

Maria Evdokimou [a], Hakam Alaqabani [a, b], Muattaz Hussain [a], and Yvonne Perrie [a]*

[a] Strathclyde Institute of Pharmacy and Biomedical Sciences, 161 Cathedral St, The University of Strathclyde, Glasgow, G4 0RE, UK.

[b] Faculty of Pharmacy, Al-Zaytoonah University of Jordan, Airport St, 11733 Amman, Jordan.

[QR for LinkedIn]

Introduction

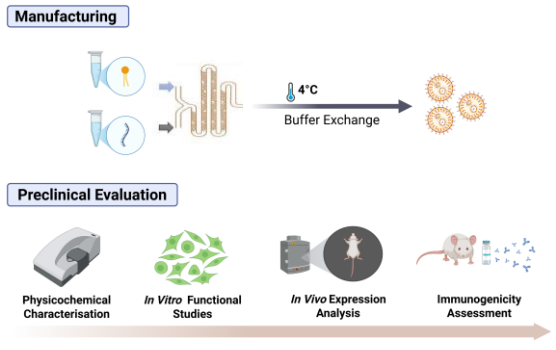
Vaccines remain a cornerstone of public health, with **mRNA-based therapeutics** representing a transformative approach to combating infectious diseases.

Lipid nanoparticles (LNPs) are essential to this platform, protecting **mRNA** cargo and facilitating cellular delivery. While ionisable lipids such as **SM-102** have been optimised for efficient mRNA encapsulation and release, recent interest has shifted toward fixed-cationic lipids like **dimethyldioctadecylammonium bromide (DDAB)**. Owing to its physicochemical stability and inherent immunostimulatory properties, DDAB holds potential to enhance both the structural integrity and immunogenicity of LNP-based vaccines.

Aim:

Enhance the **potency** and **efficiency** of LNPs by incorporating the fixed cationic lipid **DDAB** with the ionisable lipid **SM-102**, in the Moderna's COVID-19 vaccine LNP composition.

Methodology



Conclusion

- DDAB- LNPs might be **not that good delivery vehicles**, but they're **decent vaccine adjuvants**.
- **Balanced lipid ratios** are key to tailoring immune polarisation.

Results & Discussion

Physicochemical Characterisation

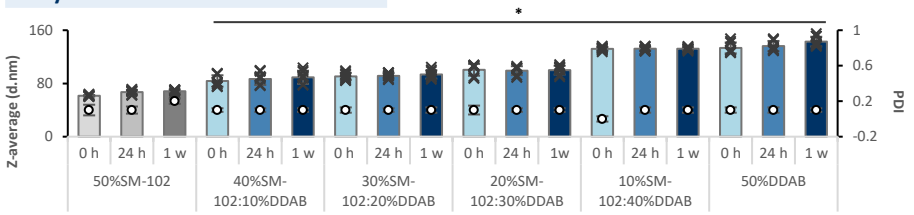


Figure 1. Physicochemical properties of DDAB-modified LNPs. LNPs were prepared via microfluidic mixing (FRR 3:1, TFR 15 mL/min, N/P 6). The aqueous phase contained Green Lantern mRNA in 50 mM citrate buffer (pH 4.0). LNPs were purified SD (n=9). Statistical significance vs. 50% SM-102 LNPs was determined by one-way ANOVA with Tukey's post-hoc test (*p < 0.05).

DSPC:Chol:	Zeta Potential (mV)	EE%	mRNA Recovery (%)
50%SM-102:1.5%DMG-PEG2000	0.0 ± 0.1	97 ± 0.2	86 ± 2
40%SM-102:10%DDAB:1.5%DMG-PEG2000	0.0 ± 0.1	98 ± 0.2	88 ± 4
30%SM-102:20%DDAB:1.5%DMG-PEG2000	-0.1 ± 0.1	100 ± 0.1	85 ± 4
20%SM-102:30%DDAB:1.5%DMG-PEG2000	0.0 ± 0.1	100 ± 0.0	78 ± 4
10%SM-102:40%DDAB:1.5%DMG-PEG2000	-0.1 ± 0.1	100 ± 0.0	81 ± 3
50%DDAB:1.5%DMG-PEG2000	0.0 ± 0.1	100 ± 0.1	89 ± 2

Table 1. Physicochemical properties of DDAB-modified LNPs. Data represent mean ± SD from three batches across three repeats (n=9). EE% = encapsulation efficiency.

Increasing DDAB content correlated with a **progressive rise** in average **particle size**, indicating a **dose-dependent effect** on LNP morphology.

In Vitro Application

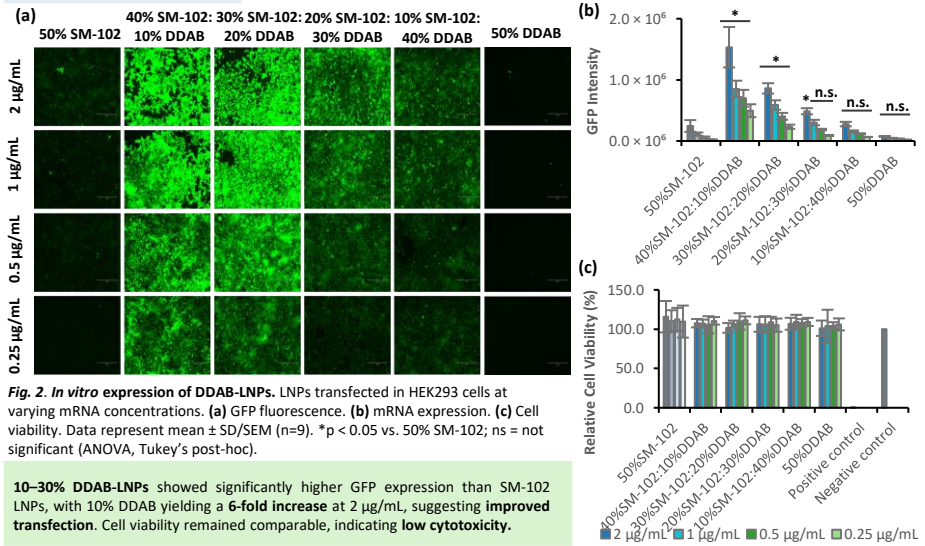


***Fig. 2. In vitro* expression of DDAB-LNPs.** LNPs transfected in HEK293 cells at varying mRNA concentrations. **(a)** GFP fluorescence. **(b)** mRNA expression. **(c)** Cell viability. Data represent mean ± SD/SEM (n=9). *p < 0.05 vs. 50% SM-102; ns = not significant (ANOVA, Tukey's post-hoc).

10–30% DDAB-LNPs showed significantly higher GFP expression than SM-102 LNPs, with 10% DDAB yielding a **6-fold increase** at 2 µg/mL, suggesting **improved transfection**. Cell viability remained comparable, indicating **low cytotoxicity.**

In Vivo Application

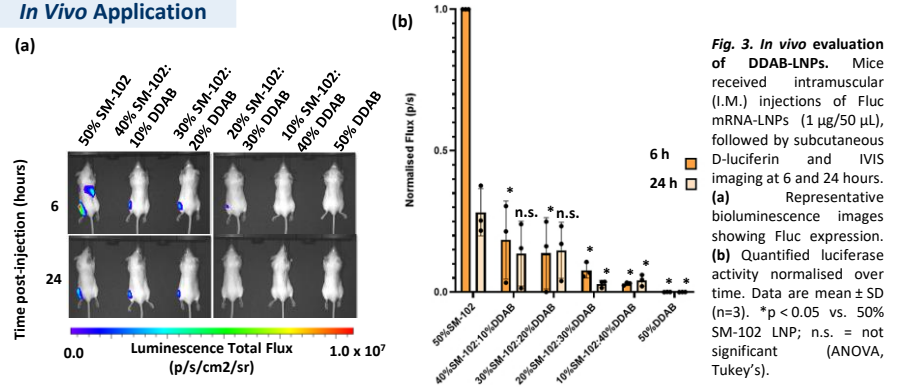


***Fig. 3. In vivo* evaluation of DDAB-LNPs.** Mice received intramuscular (I.M.) injections of Fluc mRNA-LNPs (1 µg/50 µL), followed by subcutaneous D-luciferin and IVIS imaging at 6 and 24 hours. **(a)** Representative bioluminescence images showing Fluc expression. **(b)** Quantified luciferase activity normalised over time. Data are mean ± SD (n=3). *p < 0.05 vs. 50% SM-102 LNP; n.s. = not significant (ANOVA, Tukey's).

SM-102 LNPs showed the highest expression, with **signal decreasing as DDAB content increased**. This suggests DDAB's in vitro enhancement may not fully translate in vivo, possibly due to **biodistribution** or **immune interactions** of a living organism.

Immunogenicity

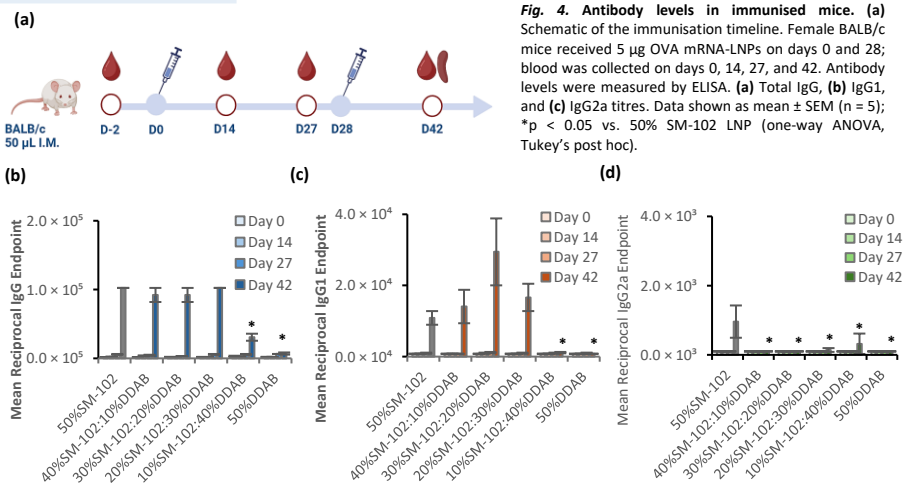


***Fig. 4.* Antibody levels in immunised mice. (a)** Schematic of the immunisation timeline. Female BALB/c mice received 5 µg OVA mRNA-LNPs on days 0 and 28; blood was collected on days 0, 14, 27, and 42. Antibody levels were measured by ELISA. **(a)** Total IgG, **(b)** IgG1, and **(c)** IgG2a titres. Data shown as mean ± SEM (n = 5); *p < 0.05 vs. 50% SM-102 LNP (one-way ANOVA, Tukey's post hoc).

All but the DDAB-rich LNPs induced **strong total IgG**. **IgG1** was elevated in **10–30% DDAB-LNPs**, slightly exceeding SM-102 controls, while **IgG2a remained low**, indicating **limited Th1-type responses**.

Acknowledgements: This work was funded by the *Intracellular Drug Delivery Centre (IDDC)* through **UK Research and Innovation (UKRI)**. It was also supported by the **EPSRC Doctoral Training Partnership (DTP)** via the *Research Training Support Grant (RTSG)*.