Tailoring Lipid Nanoparticle Composition for Better Immune Response

Muhammad Muzamil Khan[1], Ehsan Ansari Dezfouli[1], Olga Pacios Santamaria,[1], Cedric Brimacombe[2], James Russell[3], Pieter Cullis[1]

Department of Biochemistry and Molecular Biology, University of British Columbia[1], Polymorphic Bio Sciences Inc[2] , Faculty of Medicine, University of British Columbia, Canada[3]

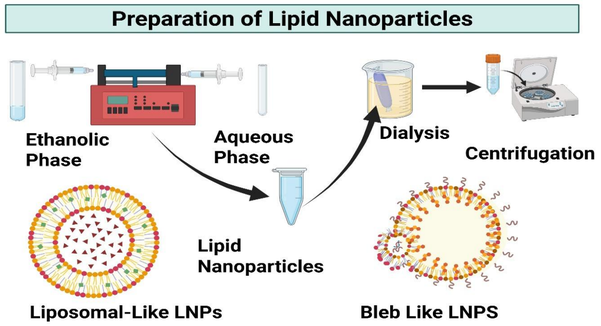
INTRODUCTION

Lipid nanoparticles (LNPs) are considered as effective mRNA delivery platforms for clinical translation, particularly due to their success in delivery of spike mRNA for COVID-19 vaccines. The mRNA based need to be delivered only to the cytoplasm to achieve desirable therapeutic effect [1]. The ionizable lipid is a crucial component of LNPs that enable encapsulation of RNA and transport to cytoplasm, while helper lipids and cholesterol provide structural stabilization and PEG-lipid enhance circulation time [2]. The change in helper lipid content leads to changes in the shape of the LNPs and variation in *in vivo* response and circulation time [3].

METHODS

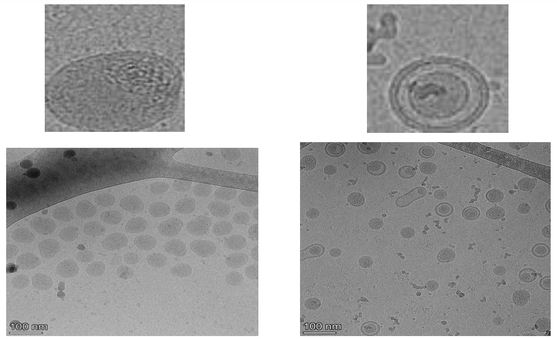
In this work, we have prepared a series of LNPs using T-mixing techniques by changing the composition of the lipid nanoparticle components. Specifically, we have changed the percentage of ionizable lipids and helper lipids within the formulation. The prepared lipid nanoparticles with varied composition were evaluated for size and zeta potential using a Malvern® Zeta Sizer and encapsulation efficiency (EE) was determined using Quant-it Ribogreen assay. The size and EE (%) was within acceptable range for all LNPs. The LNPs shape was determined using Cryo-EM Microscopy. The *in vitro* evaluation was done using bone marrow dendritic cell lines and *in vivo* immune response was evaluated using C/57 BL/6 mice by intramuscular injection of spike mRNA containing LNPs. The serum was collected after 24 hours of treatment and a single cell suspension was prepared from spleen and analyzed using flow cytometry.

PREPARATION OF LIPID NANOPARTICLES



Preparation of Lipid nanoparticles using T-Mixing Technique

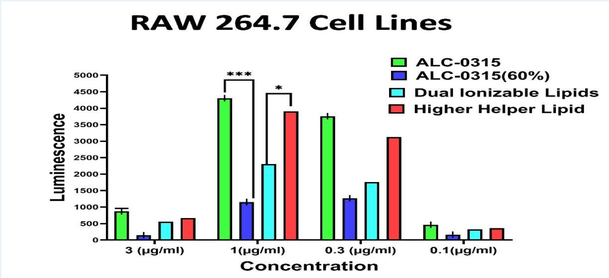
CRYO-EM IMAGES

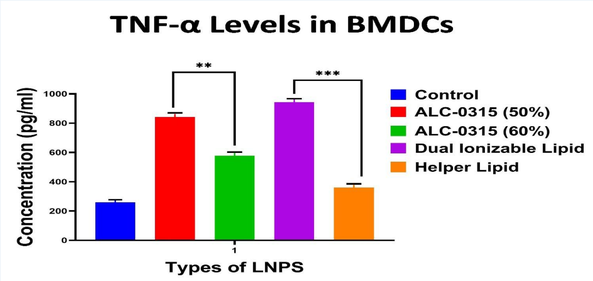


Bleb-Like LNPs **Liposomal Like-LNPs**

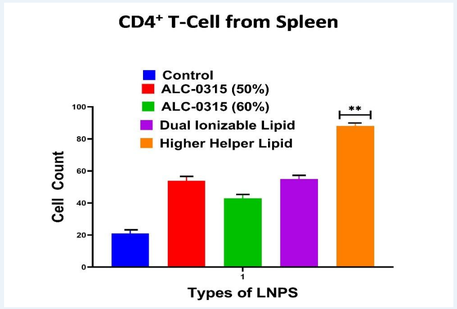
RESULTS

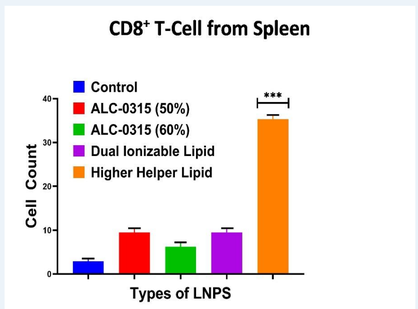
In Vitro Evaluation

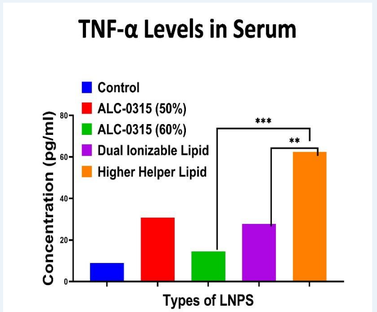




In Vivo Evaluation







CONCLUSION

The results revealed that variation in helper lipid contents lead to change in shape from bleb-like LNPs to liposomal like LNPs. The bleb-like LNPs shows better transfection potential in RAW-264.7 cell lines. On the other hand, after single IM injection of spike-mRNA, liposomal like LNPs showed better CD4+ and CD8+ count and significantly higher levels of TNF-α in serum. Further detailed immune response studies are needed to establish the effect of liposomal like LNPs in terms of immune response.

ACKNOWLEDGEMENTS

The Authors Acknowledge funding from the Canada Biomedical Research Fund and the Biosciences Research Infrastructure Fund.

REFERENCES

1. Alameh, M.-G., et al., Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. Immunity, 2021. **54**(12): p. 2877-2892. e7.

2. Lee, Y., et al., Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics. Experimental & Molecular Medicine, 2023. **55**(10): p. 2085-2096.

3. Cheng, M.H.Y., et al., Liposomal lipid nanoparticles for extrahepatic delivery of mRNA. Nature Communications, 2025. **16**(1): p. 4135.

CONTACT

Muhammad Muzamil Khan
mRNA Delivery, siRNA Delivery, Lipid Nanoparticles, Vaccine development

muzamil.khan@ubc.ca