Genetically Engineering Transfusable Platelets with mRNA-Lipid Nanoparticles is Compatible with Blood Banking Practices

Emma Kang,[1,2,3]† Colton Strong,[1,2,4]† Jerry Leung,[1,2,4,5]† Katherine E. Badior,[6] Madelaine Robertson,[1,2,4,5] Nicolas Pereyra,[2,4] Elyn Rowe,[3] Amanda Wietrzny,[6] Brenda Ma,[2,4] Zechariah Noronha,[2] Dana V. Devine,[3,4,5] Eric Jan,[4] Pieter R. Cullis,[4,5] Christian J. Kastrup[1,2,3,4,6,7*]

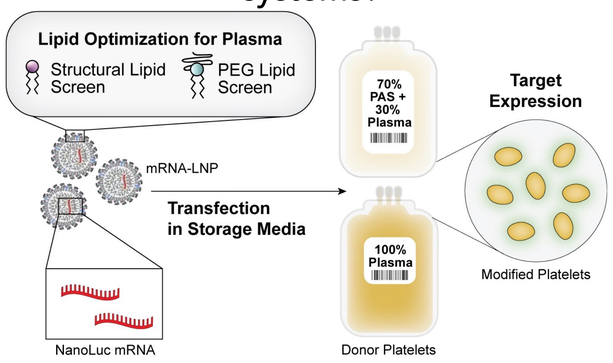
[1]Michael Smith Laboratories, University of British Columbia; Vancouver, V6T 1Z4, Canada. [2]Centre for Blood Research, University of British Columbia; Vancouver, V6T 1Z3, Canada. [3]Department of Pathology and Laboratory Medicine, University of British Columbia; Vancouver, V6T 2B5, Canada. [4]Department of Biochemistry and Molecular Biology, University of British Columbia; Vancouver, V6T 1Z3, Canada. [5]NanoMedicines Research Group, University of British Columbia; Vancouver, V6T 1Z3, Canada. [6]Blood Research Institute, Versiti Wisconsin; Milwaukee, 53226, USA. [7]Departments of Surgery, Biochemistry, Biomedical Engineering, and Pharmacology and Toxicology, Medical College of Wisconsin; Milwaukee, 53226, USA. †These authors contributed equally to this work

INTRODUCTION

Given platelets' integral role in physiology, they have the potential to be utilized as a natural delivery system if enhanced with exogenous cargo. Recently we have demonstrated that optimized lipid nanoparticles (LNPs) can be used to load cargo into platelets. However, this platform was developed using Tyrode's buffer, a crystalloid solution that is not used clinically. We present preliminary data that LNPs can be used to transfect platelets in a setting closer to a clinical environment and is a promising first step for use of enhanced platelets clinically.

BIG QUESTION

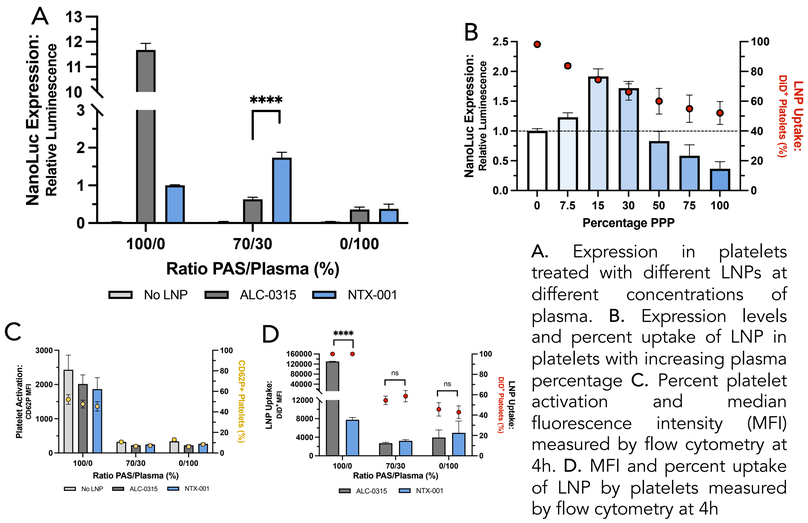
Can we engineer platelets in clinically relevant systems?



GOALS

1. Screen different lipid nanoparticles formulations for optimal delivery in plasma and plasma-based systems
2. Show mRNA-LNP treatments are scalable to high and physiological unit concentrations of platelets
3. Characterize mRNA-LNP platelets to show they are responsive to different agonists

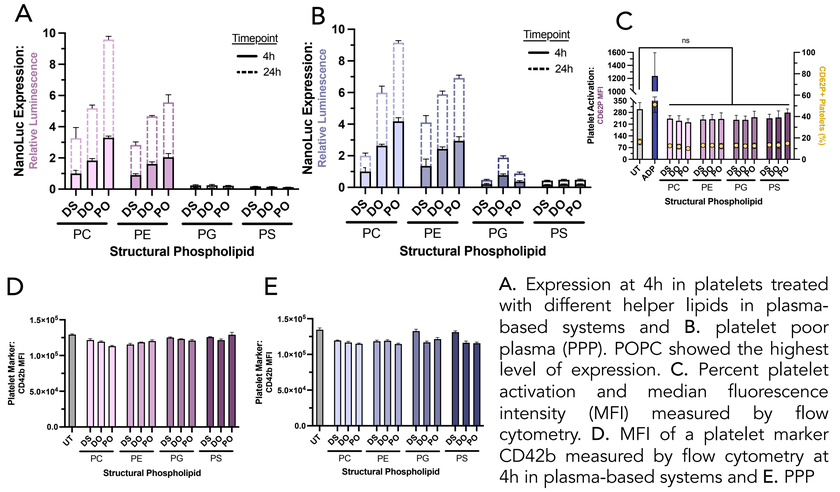
① LNPS CAN TRANSFECT PLATELETS IN PLASMA AND PLASMA-BASED SYSTEMS



A. Expression in platelets treated with different LNPs at different concentrations of plasma. B. Expression levels and percent uptake of LNP in platelets with increasing plasma percentage C. Percent platelet activation and median fluorescence intensity (MFI) measured by flow cytometry at 4h. D. MFI and percent uptake of LNP by platelets measured by flow cytometry at 4h

Key Takeaway: LNPs can be optimized for transfection of platelets in plasma

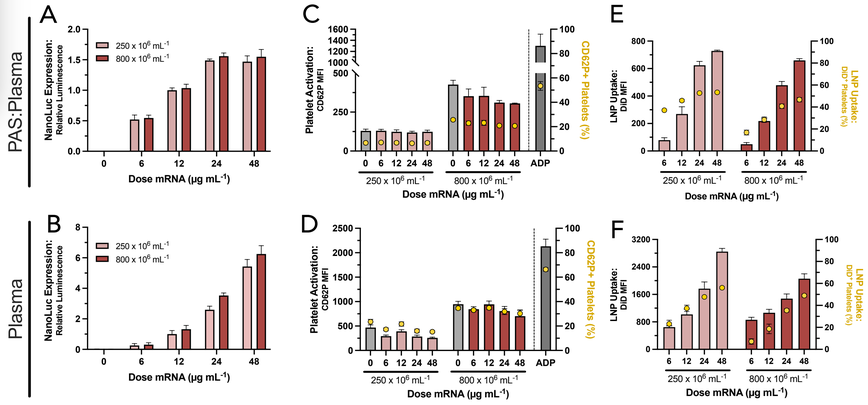
② LNPS WITH PC LIPIDS ARE MORE EFFECTIVE AT TRANSFECTING PLATELETS



A. Expression at 4h in platelets treated with different helper lipids in plasma-based systems and B. platelet poor plasma (PPP). POPC showed the highest level of expression. C. Percent platelet activation and median fluorescence intensity (MFI) measured by flow cytometry. D. MFI of a platelet marker CD42b measured by flow cytometry at 4h in plasma-based systems and E. PPP

Key Takeaway: Transfection in plasma and plasma-based systems requires LNPs formulated with PC helper lipids

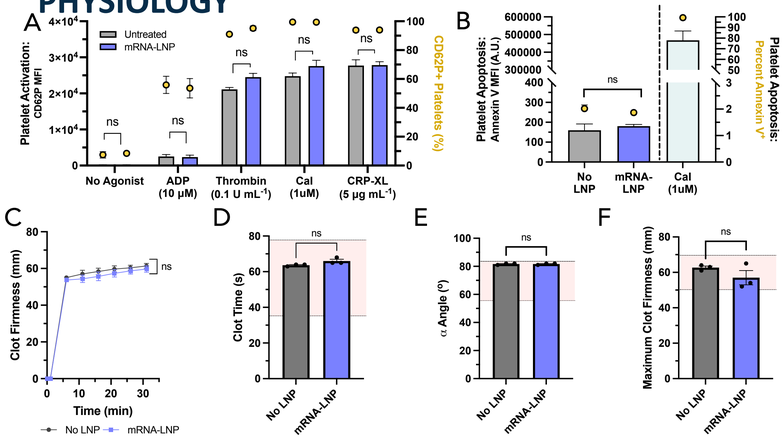
③ LNP TREATMENTS ARE SCALABLE



A. Expression in platelets treated with different doses of LNP at different concentrations in plasma-based systems and B. platelet poor plasma (PPP). C. Percent platelet activation and median fluorescence intensity (MFI) measured by flow cytometry in plasma-based systems D. in PPP. E. Percentage of LNP uptake and MFI measured by flow cytometry in plasma-based systems and F. in PPP

Key Takeaway: mRNA-LNP transfection is scalable to high and physiological unit concentrations in plasma and plasma-based systems

④ LNP TRANSFECTED PLATELETS HAVE NORMAL PHYSIOLOGY



A. Platelet activation and median fluorescence intensity (MFI) after activation with ADP, thrombin, calcium ionophore (Cal), and CRP-XL measured by flow cytometry. B. Platelet apoptosis MFI and percent measured by Annexin V by binding to phosphatidylserine using flow cytometry. C-F. ROTEM measurements measuring clot firmness overtime (C), clot time (D), alpha angle (E), and maximum clot firmness (F). Red shaded areas represent clinically acceptable ranges

Key Takeaway: LNP treated platelets are agonist responsive, and the treatments do not affect their coagulability morphology

CONCLUSION

LNP formulations can transfect platelets in clinically relevant systems and do not affect their innate physiology

ACKNOWLEDGMENTS

I would like to thank C. Strong, and J. Leung for helpful insights and discussion and data contributions We would also like to thank the Canadian Blood Services and the blood donors for providing the pooled platelet products.

CONTACT INFORMATION

Emma Kang | MSc Candidate
Centre for Blood Research | Devine and Kastrup Lab
University of British Columbia, Vancouver, BC, Canada
Unceded xʷməθkʷəy̓əm (Musqueam) territory
Please direct any enquiries to E. Kang at emma.kang@msl.ubc.ca