Optimized Lipid Nanoparticles Enable Exogenous Protein Expression in Platelets

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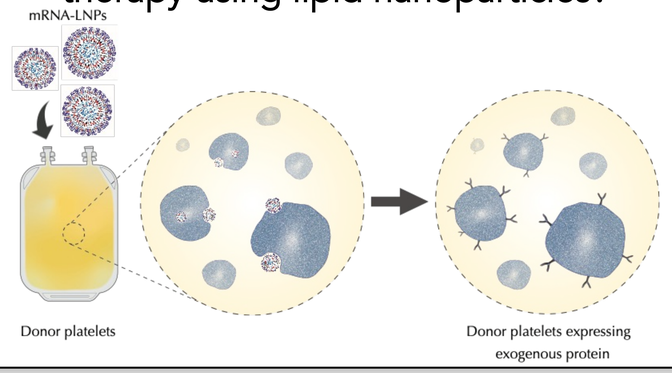
INTRODUCTION

Platelet transfusions are an integral treatment for managing bleeding and thrombocytopenia. Although anucleate, mature platelets synthesize protein de novo during circulation and storage, making them amenable to mRNA gene therapy; **however, there remains to be an effective transfection technique.** Given the role of platelets in hemostasis and disease, enhanced platelets loaded with exogenous therapeutic protein could expand donor platelet transfusions as a cell therapy in a variety of clinical indications.

BIG QUESTION

Can platelets be functionalized *ex vivo* as a cell therapy using lipid nanoparticles?



GOALS

1. Achieve Expression of Exogenous Proteins in Donor Platelets using LNPs
2. Identify platelet optimized LNP formulation by testing different helper and ionizable lipids
3. Determine if LNP treated platelets retain hemostatic function *in vitro*

ACKNOWLEDGMENTS

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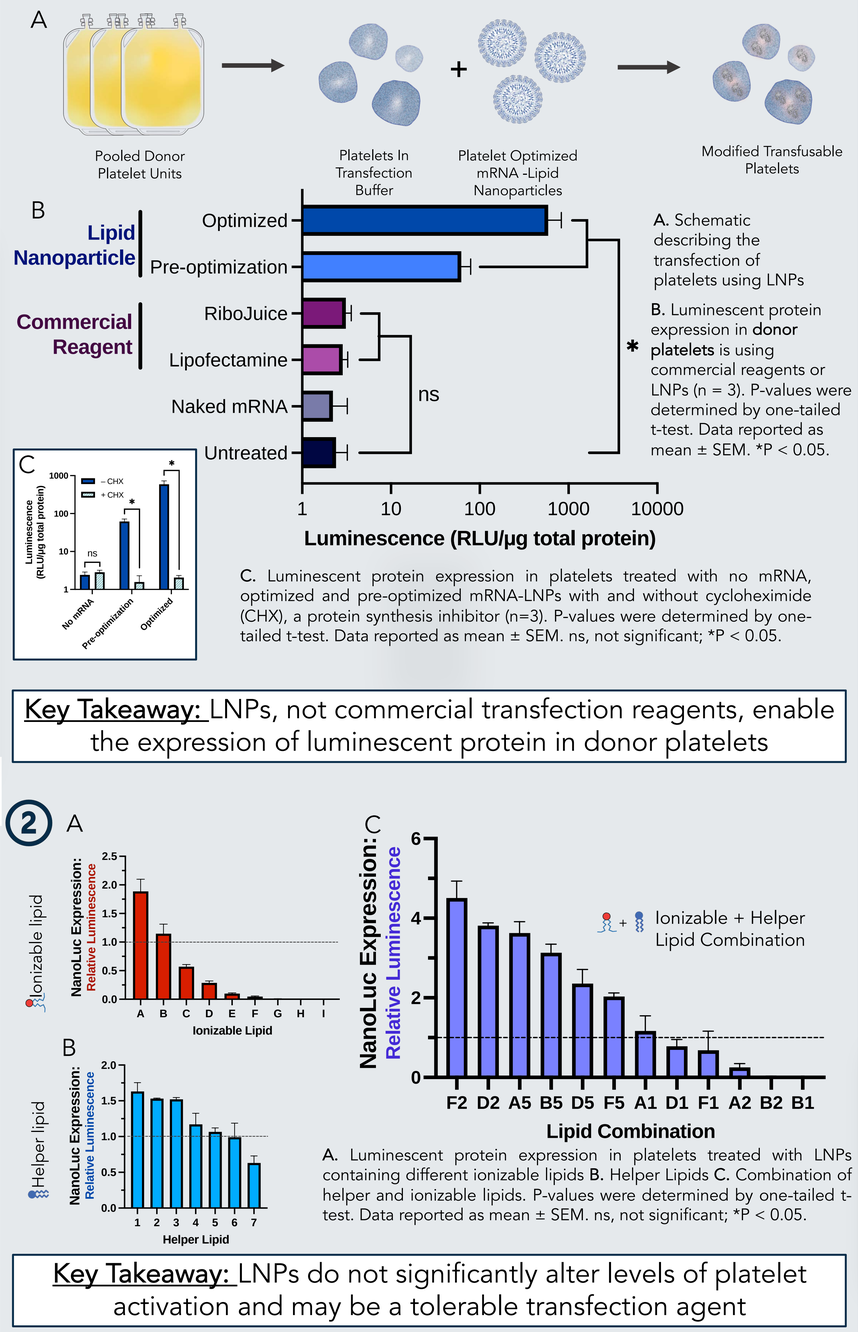
① LNP ENGINEERED PLATELETS EXPRESS PROTEIN



A. Schematic describing the transfection of platelets using LNPs

B. Luminescent protein expression in **donor platelets** is using commercial reagents or LNPs (n = 3). P-values were determined by one-tailed t-test. Data reported as mean ± SEM. *P < 0.05.

C. Luminescent protein expression in platelets treated with no mRNA, optimized and pre-optimized mRNA-LNPs with and without cycloheximide (CHX), a protein synthesis inhibitor (n=3). P-values were determined by one-tailed t-test. Data reported as mean ± SEM. ns, not significant; *P < 0.05.

Key Takeaway: LNPs, not commercial transfection reagents, enable the expression of luminescent protein in donor platelets

② LNP ENGINEERED PLATELETS EXPRESS PROTEIN

A. Luminescent protein expression in platelets treated with LNPs containing different ionizable lipids **B.** Helper Lipids **C.** Combination of helper and ionizable lipids. P-values were determined by one-tailed t-test. Data reported as mean ± SEM. ns, not significant; *P < 0.05.

Key Takeaway: LNPs do not significantly alter levels of platelet activation and may be a tolerable transfection agent

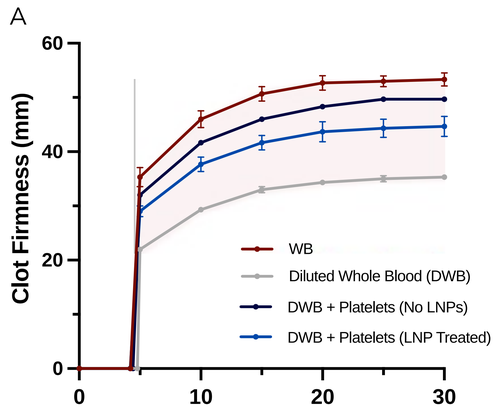
REFERENCES

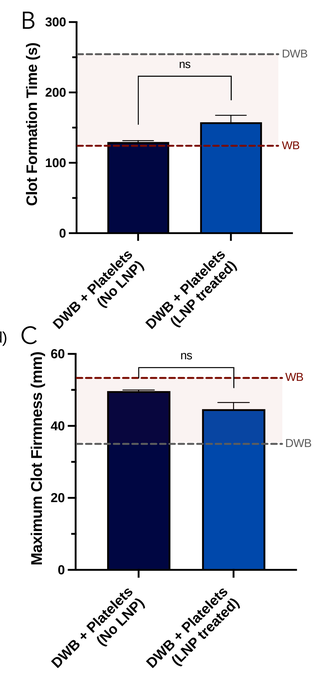
1. **Kulkarni J-A et al.** Lipid nanoparticle technology for clinical translation of siRNA therapeutics. *Acc Chem Res 2019; 52(9);: 2435-2444*
2. **Leung, J., et al.** Emerging gene therapies for enhancing the hemostatic potential of platelets. *Transfusion 2021;* 61(1);: S275-S285.

③ LNP TREATED PLATELETS ARE FUNCTIONAL

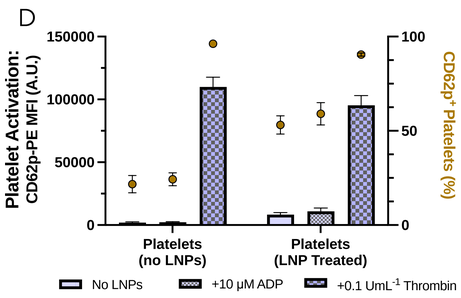
To test coagulability of LNP treated platelets: Rotational Thromboelastometry

In vitro measure of clot stiffness





A. Representative rotational thromboelastometry (ROTEM) clot firmness over time, with clotting initiated by ellagic acid (n=3). The red shaded region is the area between whole blood (WB) "normal clotting" and diluted WB (DWB) "impaired clotting" without the addition of platelets. The blue curves are each DWB with platelets added, with and without previous LNP treatment. **B** and **C.** Quantifying ROTEM parameters clot formation time (B) and maximum Clot Firmness (C). The dashed lines represent firmness of WB (dark red) and DWB (light grey). P-values were determined by one-way ANOVA. Data reported as mean ± SEM. ns, not significant.



D. Platelet CD62p surface of platelets activated with ADP or thrombin shows that LNP treated platelets are still agonist responsive. Quantification of median fluorescence intensity (MFI) and percentage of platelets (yellow markers, right y-axis) positive for CD62p (n = 3).

Key Takeaway: LNP treated platelets maintain their ability to activate and contribute to growth and stiffness of blood clots

CONCLUSION

LNPs enable the expression of exogenous protein in donor platelets and do not impair platelet function *in vitro*

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