Expression of exogenous proteins in donor platelets using optimized lipid nanoparticles and mRNA

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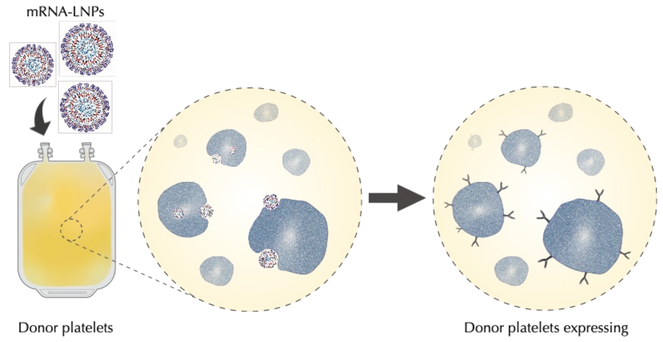
INTRODUCTION

Platelet transfusions are an essential treatment for attenuating bleeding but are often ineffective in cases of intractable hemorrhage. 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. Advancements in lipid nanoparticle (LNP) technology has enabled leading COVID vaccines and is an efficient method to deliver nucleic acids into target cells. **Recently, our team developed a LNP approach to successfully express exogenous enzymes in platelets**, a first step towards demonstrating that platelet coagulability could be engineered. Here, we describe how we optimized LNPs containing mRNA (mRNA-LNPs) to enable exogenous protein expression in platelets *ex vivo*. Within the library of mRNA-LNPs tested, exogenous protein expression did not require, nor correlate with, platelet activation. LNP engineered platelets retained hemostatic function and agonist responsiveness *in vitro* and controlled bleeding comparably to unmodified platelets after transfusion into coagulopathic rats. We are now using this technology to express proof-of-concept proteins to deliberately alter platelet function. Further development of this technology will lead to more effective platelet therapies.

BIG QUESTION

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

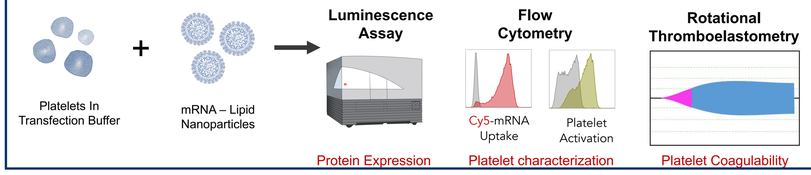


GOALS

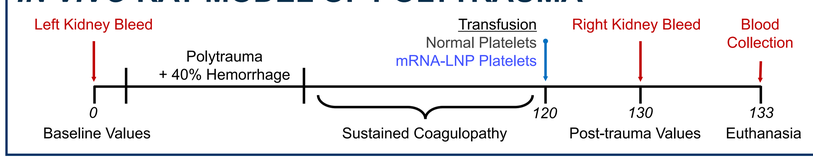
1. Identify platelet-optimized LNPs to achieve the expression of exogenous protein in platelets
2. Determine if LNP transfection adversely affects platelet function *in vitro* and *in vivo*

METHODOLOGY

IN VITRO ANALYSIS



IN VIVO RAT MODEL OF POLYTRAUMA



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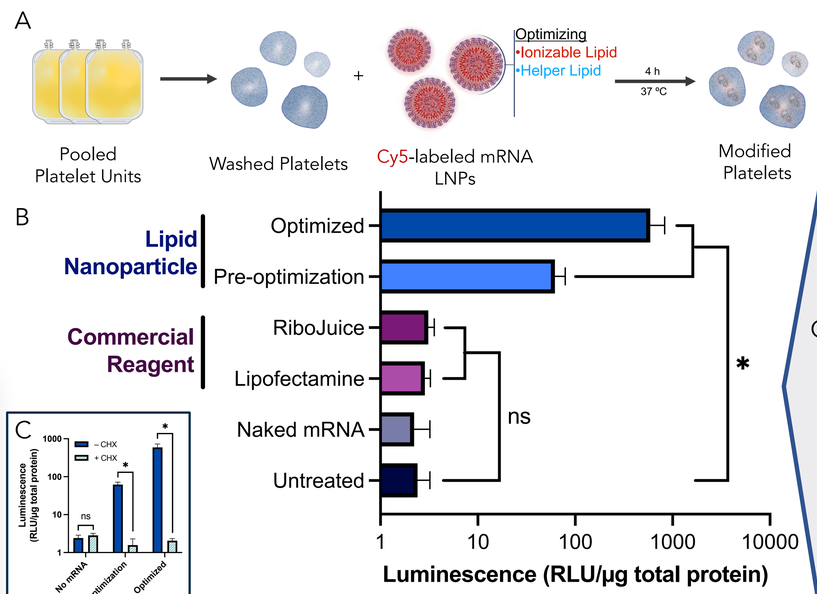
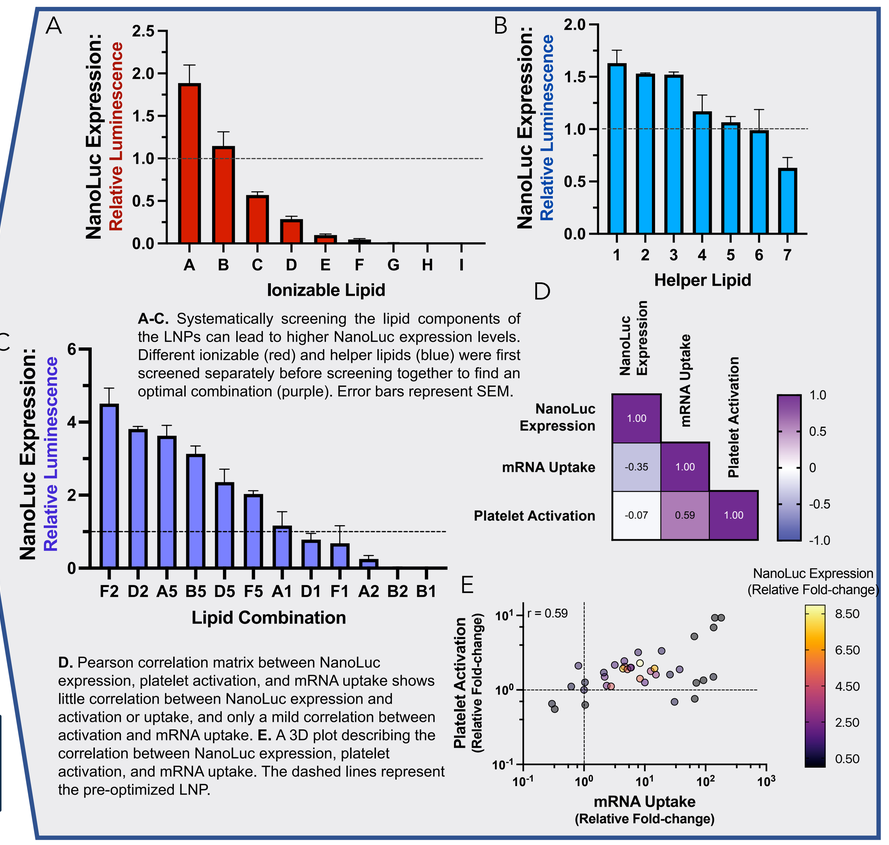
RESULTS

① PLATELET-OPTIMIZED LNP ENABLE EXOGENOUS PROTEIN EXPRESSION



A. Schematic describing the transfection of platelets using LNPs **B.** Luminescent protein expression in donor platelets is using commercial reagents or mRNA-LNPs (n = 3). P-values were determined by one-tailed t-test. **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: Platelet-optimized LNP, not commercial transfection reagents, enable the expression of luminescent protein in donor platelets and expression does not correlate with platelet activation

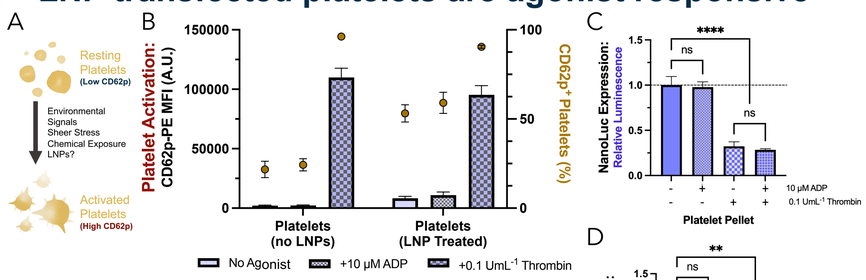


A-C. Systematically screening the lipid components of the LNPs can lead to higher NanoLuc expression levels. Different ionizable (red) and helper lipids (blue) were first screened separately before screening together to find an optimal combination (purple). Error bars represent SEM.

D. Pearson correlation matrix between NanoLuc expression, platelet activation, and mRNA uptake shows little correlation between NanoLuc expression and activation or uptake, and only a mild correlation between activation and mRNA uptake. **E.** A 3D plot describing the correlation between NanoLuc expression, platelet activation, and mRNA uptake. The dashed lines represent the pre-optimized LNP.

② LNP TRANSFECTED PLATELETS RETAIN FUNCTION *IN VITRO* AND *IN VIVO*

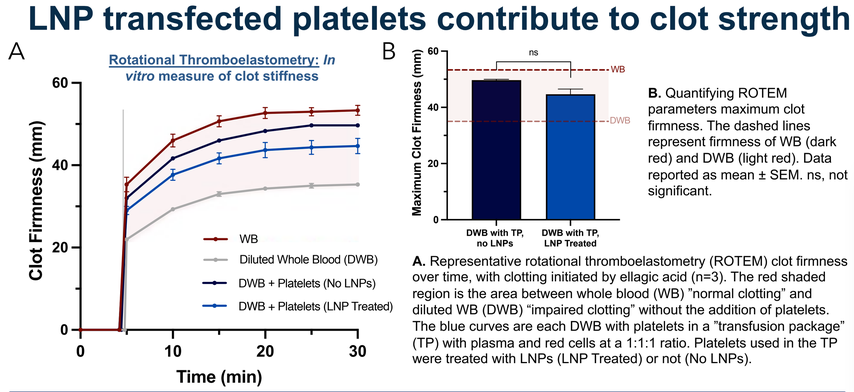
LNP transfected platelets are agonist responsive



A. Schematic describing platelet activation and association with CD62p surface exposure detectable by flow cytometry **B.** CD62p surface expression on platelets activated with ADP or thrombin shows that LNP treated platelets are agonist responsive. Quantification of median fluorescence intensity (MFI) and percentage of platelets (yellow markers, right y-axis) positive for CD62p (n = 3). **C-D.** mRNA-LNP treated platelets – activated with 10 µM ADP, 0.1 UmL^{-1} thrombin, or both – do not yield increased luminescence relative to those not stimulated with any agonists (represented by the dashed line), but instead yield less luminescence in both the platelet pellet (C) and supernatant (D). Error bars represent SEM. ns, not significant; **P < 0.01; ***P < 0.001.

Key Takeaway: LNP treated platelets are responsive to agonists ADP and thrombin and exogenous translation is independent of agonist stimulation

LNP transfected platelets contribute to clot strength

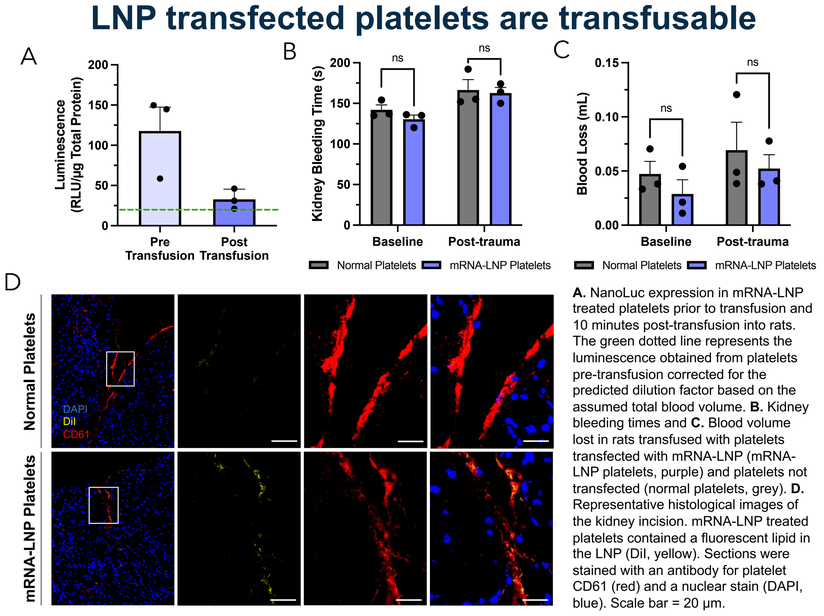


A. Representative rotational thromboelastometry (ROTEM) clot firmness over time, with clotting initiated by ellagic acid (n=3). The red shaded region is the area between whole blood (WB) "normal clotting" and diluted WB (DWB) "impaired clotting" without the addition of platelets. The blue curves are each DWB with platelets in a "transfusion package" (TP) with plasma and red cells at a 1:1:1 ratio. Platelets used in the TP were treated with LNPs (LNP Treated) or not (No LNPs).

B. Quantifying ROTEM parameters maximum clot firmness. The dashed lines represent firmness of WB (dark red) and DWB (light red). Data reported as mean ± SEM. ns, not significant.

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

LNP transfected platelets are transfusable



A. NanoLuc expression in mRNA-LNP treated platelets prior to transfusion and 10 minutes post-transfusion into rats. The green dotted line represents the luminescence obtained from platelets pre-transfusion corrected for the predicted dilution factor based on the assumed total blood volume. **B.** Kidney bleeding times and **C.** Blood volume lost in rats transfused with platelets transfected with mRNA-LNP (mRNA-LNP platelets, purple) and platelets not transfected (normal platelets, grey). **D.** Representative histological images of the kidney incision. mRNA-LNP treated platelets contained a fluorescent lipid in the LNP (DiI, yellow). Sections were stained with an antibody for platelet CD61 (red) and a nuclear stain (DAPI, blue). Scale bar = 20 µm.

Key Takeaway: LNP treated platelets are transfusable in vivo and accumulate in wound sites

CONCLUSION

Platelet-optimized LNPs enable the expression of exogenous proteins in donor platelets and do not impair platelet function *in vitro* and *in vivo*

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