79 Virtual VANCOUVER NANOMEDICINE DAY

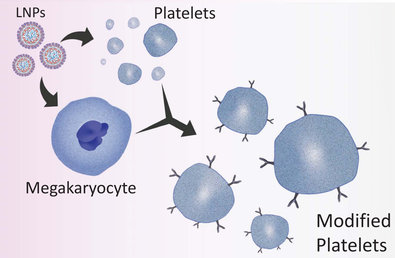
September 17th, 2020

University of British Columbia
Vancouver, BC

Enhancing Platelets with Gene Therapy for More Effective Transfusions

Jerry Leung[1,2], Andrew Hagner[3], Dominik Witzigmann[1], Lih Jiin Juang[1,2], Peter Zandstra[2,3], Pieter Cullis[1], Christian Kastrup[1,2]

[1] University of British Columbia, Biochemistry and Molecular Biology, Vancouver, Canada
[2] University of British Columbia, Michael Smith Laboratories, Vancouver, Canada
[3] University of British Columbia, School of Biomedical Engineering, Vancouver, Canada



INTRODUCTION

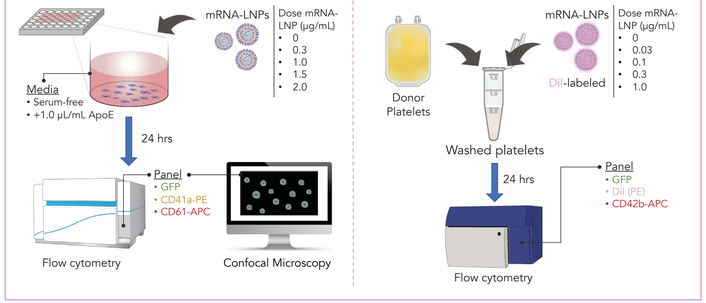
Platelets are small, enucleate, multifunctional cells that modulate many important physiological roles including hemostasis and immune signalling. Currently transfused as a therapy to maintain hemostatic balance, platelets are an integral part of hemorrhage management. However, transfusions can be ineffective in the most severe cases of hemorrhage. In addition to hemostasis, platelets are a potential cell therapy in other applications, but development has been hindered by inadequate methods to control which proteins are expressed by platelets. For example, there are no methods to express exogenous proteins in transfusable platelets, which would expand their use to help treat the diseases they modulate. Current methods to modify platelets largely entail viral transduction of megakaryocytes (MKs), whereby proteomic changes in the MK are inherited by the resulting platelets. The use of viruses, however, present challenges in efficiency and scale-up for clinical use. **Another method that is easily amenable to scale-up for the clinic is therefore needed to produce modified, transfusable platelets, and thus enhance their protein composition for specific applications.**

AIM

To develop methods needed to produce modified platelets by **directly transfecting donor-derived platelets and megakaryocytes (MKs) with mRNA via lipid nanoparticle (LNP)-mediated delivery.** LNPs have already demonstrated clinical safety and efficacy for gene therapy[1,2], and cultured MKs provide an alternative source of cells that can be engineered to produce modified platelets[3]. It is therefore hypothesized that, as an initial proof-of-concept, LNPs containing mRNA encoding for GFP can be delivered to MKs and platelets to engender GFP expression in both cell types, which can ultimately be extended towards other proteins to enhance the natural coagulability and functional repertoire of platelets.

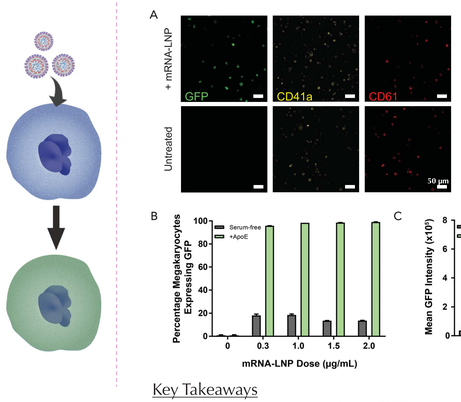
METHOD

- Lipid nanoparticles containing mRNA (mRNA-LNPs) encoding for GFP were formulated and used to treat both megakaryocytes and platelets at varying mRNA-LNP doses.
  - *For megakaryocytes:* The MKs, derived from cord-blood hematopoietic stem cells (HSCs), were treated with the mRNA-LNPs with or without 1 μg/mL of apolipoprotein E (ApoE) to facilitate LNP uptake.
  - *For platelets:* Donor platelets were washed and treated with the mRNA-LNPs but incorporating the lipophilic tracer, DiI, to track particle uptake alongside expression.



RESULTS

1. Megakaryocytes are readily transfected and express GFP



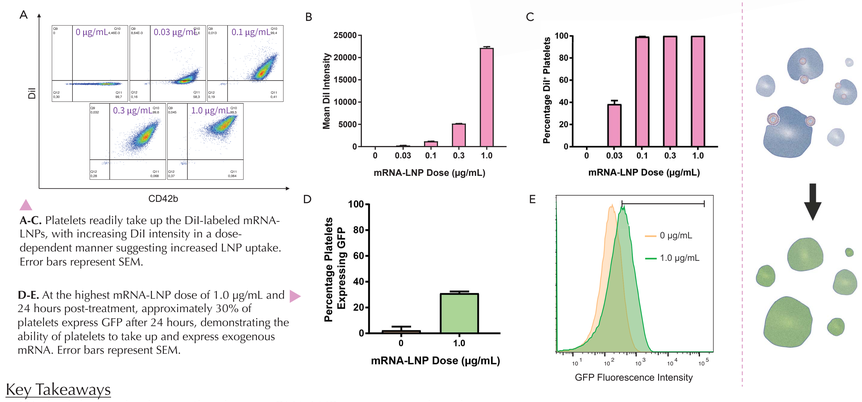
A. Confocal microscopy images of megakaryocytes treated with 1.0 μg/mL LNP (top) supplemented with 1.0 μg/mL ApoE and labeled with the megakaryocyte-specific cell markers, CD41a and CD61, demonstrate GFP expression and successful transfection, as compared with the untreated cells (bottom).

B–D. Nearly all megakaryocytes express GFP when treated with as low a dose of 0.3 μg/mL mRNA-LNPs and supplemented with 1.0 μg/mL ApoE, with increased GFP fluorescence intensity above 1.0 μg/mL of mRNA-LNPs. Megakaryocytes were identified as those positive for the CD41a and CD61. Error bars represent SEM.

Key Takeaways

- Megakaryocytes from cord-blood HSCs are easily transfected *in vitro* with mRNA-LNPs with high efficiency
- mRNA-LNP dose of 1.0 μg/mL appears optimal for high transfection efficiency and protein production
- *Next step:* Verify that platelets produced also inherit changes and express physiologically-relevant protein

2. Platelets readily take up mRNA-LNPs to express GFP



A-C. Platelets readily take up the DiI-labeled mRNA-LNPs, with increasing DiI intensity in a dose-dependent manner suggesting increased LNP uptake. Error bars represent SEM.

D-E. At the highest mRNA-LNP dose of 1.0 μg/mL and 24 hours post-treatment, approximately 30% of platelets express GFP after 24 hours, demonstrating the ability of platelets to take up and express exogenous mRNA. Error bars represent SEM.

Key Takeaways

- Washed donor platelets easily take up mRNA-LNPs even at very low doses
- Higher mRNA-LNPs doses can lead to expression of exogenous mRNA
- *Next steps:* Optimize LNPs for higher mRNA expression levels, and express physiologically-relevant proteins

CONCLUSIONS

- **Megakaryocytes and platelets can readily be transfected with LNPs to produce exogenous protein.**
- The production of modified platelets *in vitro* as a result of the modified megakaryocytes will need to be verified, with resulting platelets extensively characterized to determine changes in physiologic functions.
- The difference between LNP uptake and expression of delivered mRNA in *ex vivo*-treated donor platelets also suggests that some of the mRNA may still be sequestered or inaccessible to translation.
  - Further optimization of different LNP formulations and treatment processes may allow for a higher percentage of platelets expressing the exogenously delivered mRNA.
- If all is successful, the modified platelets can be transfused into animal models to determine their efficacy as a novel treatment towards various platelet-related diseases. Alternatively, modified megakaryocytes can be transfused to produce modified platelets directly in the body.
- **Eventually, this can lead to the creation of a platform technology that in the long-term will allow platelets to deliver therapeutic proteins and yield more effective platelet products.**

ACKNOWLEDGEMENTS

J. Leung is supported by the Frederick Banting and Charles Best Canada Graduate Scholarships Doctoral Award. D. Witzigmann acknowledges support from the Swiss National Science Foundation. We would also like to thank the Canadian Blood Services and the blood donors for providing the pooled platelet products.

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. **Akinc A-M et al**. The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. *Nat Nanotechnol 2019; 14(12);: 1084-1087*
3. **Ito Y et al**. Turbulence activates platelet biogenesis to enable clinical scale ex vivo production. *Cell 2018; 174;: 638-648*

CONTACT INFORMATION

Jerry Leung | PhD Candidate
Department of Biochemistry and Molecular Biology
University of British Columbia, Vancouver, BC, Canada

Please direct any enquiries to Jerry Leung at jerry.leung@msl.ubc.ca.

Collaborations involving the megakaryocytes, platelets, or lipid nanoparticles are all welcome!