MEG-01 cells generate particles with platelet functionality: Development of an *in vitro* platelet model

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Background

Throughout the COVID-19 pandemic, the civilian medical sector encountered a critical blood shortage, particularly in platelet donations. Platelets, the smallest blood cells in the human body, are responsible for clotting blood, and play an important role in maintaining hemostasis. For major traumatic events and penetrating injuries, platelet transfusion units are often needed to assist in blood clot formation. However, the short shelf life of platelet transfusion units and critical shortage in the civilian sector prompted a desperate need to research and improve units to allow for a longer shelf life. However, platelet research requires donor platelets, resulting in even further stress on the already critical shortage. In this poster, a human megakaryoblast leukemia cell line, MEG-01, was developed and matured into platelet-like particles. It is the hope that these platelet-like particles can survive and perform similarly in functional assays to that of human platelets. Confirmation of their similar activation and behavioral patterns would allow for the study of platelets and platelet units without the need for human donors.

Methods

Generation of MEG-01 Cell-derived Platelet-like Particles

MEG-01 megakaryoblasts (ATCC, CRL-2021) were induced to generate platelet-like particles (PLP) which were purified and harvested as described by Persson, et al (2021). Briefly, MEG-01 cells were treated with either recombinant human thrombopoietin (TPO) or phorbol myristate acetate (PMA) for 72 hours. Induced PLPs were size-selected to obtain TPO-induced platelet-like particles (TPO-PLP) or PMA-induced PLP (PMA-PLP).

Human Platelet Preparation

With approval from the Penn Institutional Review Board, blood from healthy volunteers was collected into either sodium citrate tubes or tubes containing no anti-coagulant. For aggregometry and Oroboros studies, citrated blood was centrifuged at low speed to obtain platelet rich plasma (PRP) then further spun at higher force to obtain platelet poor plasma. For calcium mobilization assays, two anticoagulants- apixaban and PPACK- were quickly added to the blood collected with no anti-coagulant. PRP was then obtained following a low force spin.

96-well Plate Aggregometry

Aggregometry was performed as per Chan et al. (2018) with modifications. PRP or PLP-rich media were combined with collagen and absorbance measurements (at 600 nm) were taken using the Synergy HTX multi-mode plate reader (BioTek Instruments).

Calcium Mobilization

PRP, TPO-PLP, and PMA-PLP were pre-incubated with the fluorescent Ca^{2+}-indicator dye, Fluo-4 NW (ThermoFisher Sci) and added to wells of a black 96-well plate containing five different platelet activation agonists. Fluorescence was measured with Synergy HTX plate reader (BioTek Instruments).

Mitochondrial Respiration

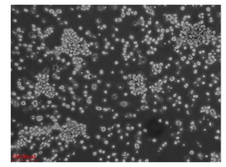
Mitochondrial respiratory function was measured using the Oroboros high-resolution oxygraphy system.

Results

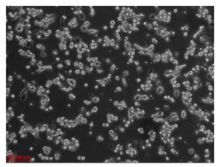
MEG-01 Viability

	Average Cell Size (um)	Cell Count (cell/mL)	Viability Percentage (%)
TPO	18.105	1495000	90%
PMA	17.275	879500	77%

Average cell size, count, and percent alive of TPO-PLP and PMA-PLP after the maturation process.

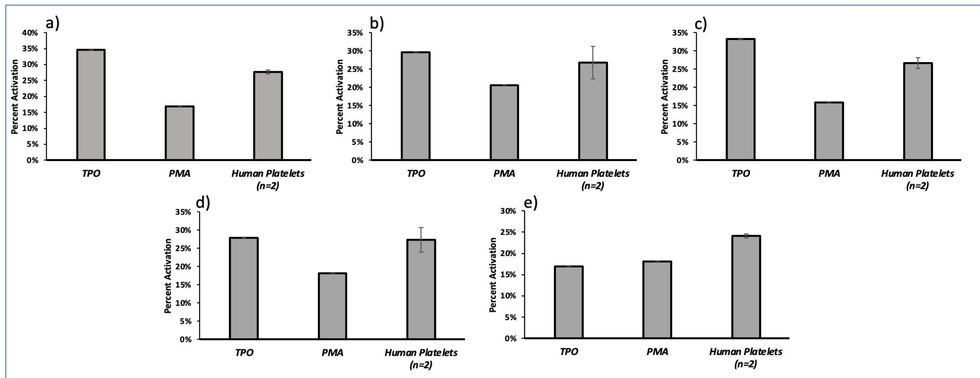


TPO Day 3



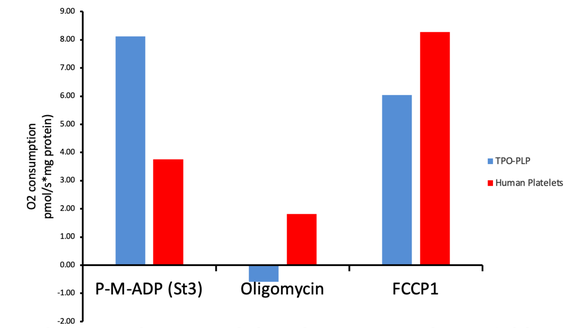
PMA Day 3

As the MEG-01 cells matured, it was seen that cells maturing with TPO developed and grew in large colonies, whereas cells maturing in PMA developed and grew either as single cells or smaller colonies.



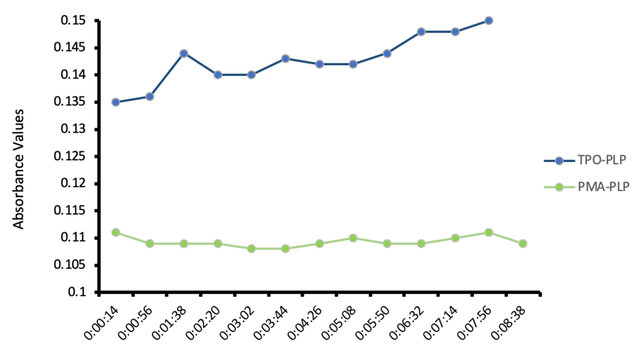
TPO-PLPs show a Ca^{2+} mobilization response similar to that of platelets from healthy volunteers.

Platelet activation (shown as percent activation) determined fluorescently after introducing the platelet receptor agonists: a) Convulxin (CVX), b) 9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin F2α (U46619), c) Thrombin Receptor Activator Peptide 6 (TRAP-6), d) AY-NH_2, and e) Adenosine 5'-diphosphate Sodium Salt (ADP). Ca^{2+} influx was measured in TPO-PLP, PMA-PLP, and healthy human platelets (*n=2*)



Preliminary data reveal that the TPO-PLPs have viable mitochondria that behave similarly to freshly isolated healthy platelets.

We found that the TPO-PLPs are consuming O_2 and respond to mitochondrial substrates and inhibitors to generate coupled respiration (state 3), uncoupled respiration (oligomycin), and maximum respiration (FCCP).



TPO-PLPs, but not PMA-PLPs, can aggregate in response to collagen treatment.

With TPO-PLP, the aggregation was sustained after 8 minutes, but the PMA-PLP did not show any response. In healthy human platelets, the aggregation rates are much higher and have a higher absorbance value (data not shown). A higher absorbance value tends to be indicative of stronger activation. However, it is currently unknown if the stronger absorbance value is a result of a larger concentration of platelets/mL in the human sample or stronger aggregation behavior.

Summary and Conclusions

- PLP derived from TPO treatment showed higher and more viable yields than those from PMA.
- TPO-PLP showed a pattern of Ca^{2+} mobilization similar to that of healthy human platelets. In both, fluorescent levels increased in response to treatment with five different receptor agonists. The response in PMA-PLP was consistently lower.
- TPO-PLP aggregation was robust in response to the activator, collagen. However, PMA-derived PLPs did not show a response to collagen.
- In an early study, TPO-PLPs appear to have viable mitochondria that behave similarly to freshly harvested healthy platelets. More experiments are necessary to confirm this finding.
- TPO-induced PLP are healthier and perform better on platelet functional assays than PLP derived from PMA treatment.

- **Our data from multiple functional assays support our development of our MEG-01 PLP-generating system for modeling human platelets.**

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Acknowledgements

Thank you to the College Alumni Society, especially Dr. Louis H Castor, C'48, and CURF for help funding this project.

References

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