Optimizing platelet secretome production: impact of prostaglandin I_2 during platelet isolation on platelet activation and secretome molecular composition

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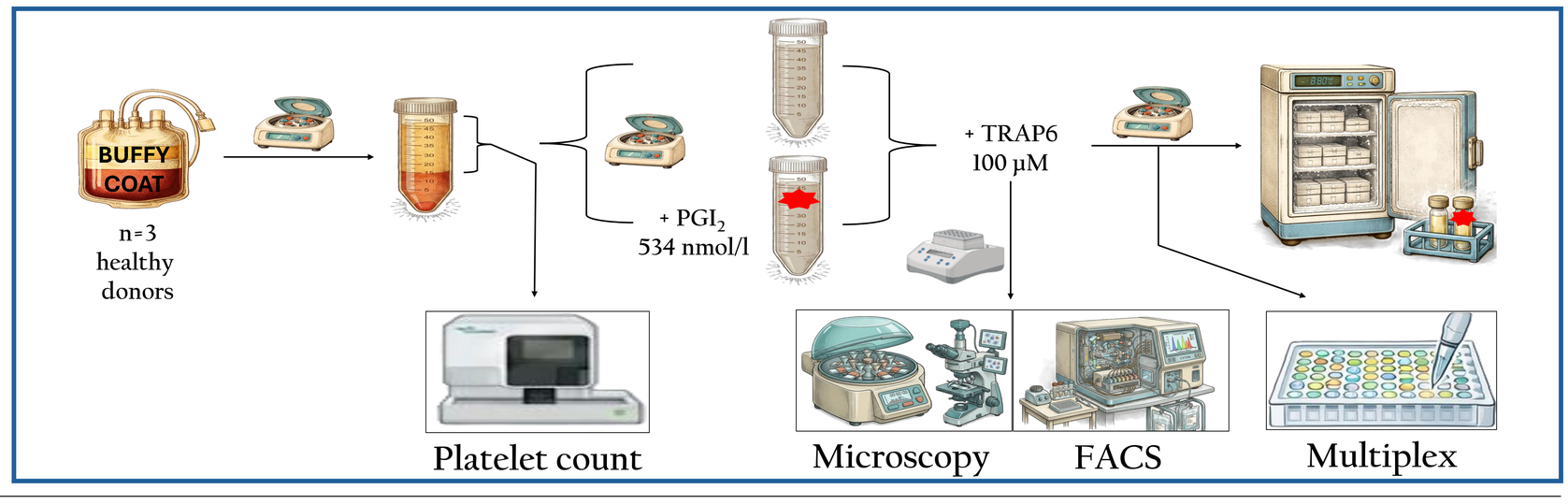
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Background

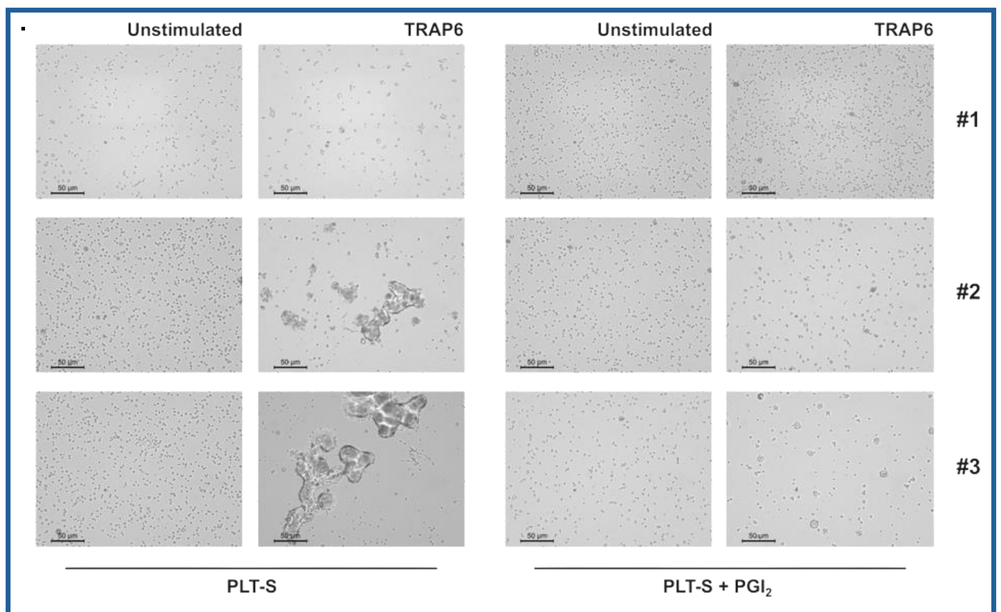
Platelet secretomes (PLT-S) are emerging as promising therapeutic bioproducts for regenerative medicine and immunomodulation. However, methodological variability during platelet isolation and activation limits reproducibility and functional consistency. Prostaglandin I_2 (PGI_2) is commonly used to maintain platelet quiescence during processing, yet its impact on subsequent platelet responsiveness and the molecular profile of the obtained secretomes remains poorly characterized.

Methods

- Washed platelets were isolated from buffy coats of healthy donors (n=3) in the absence (PLT-S) or presence of PGI_2 (PLT-S+PGI_2).
- Platelets were activated with TRAP-6.
- Platelet activation and degranulation were assessed by microscopy and by flow cytometry.
- Secretome composition was quantified using multiplex technology.
- Paired *t*-test statistical analyses were applied.

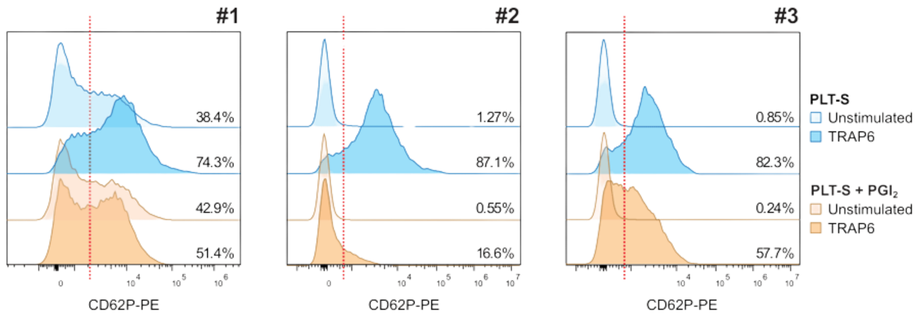


Results



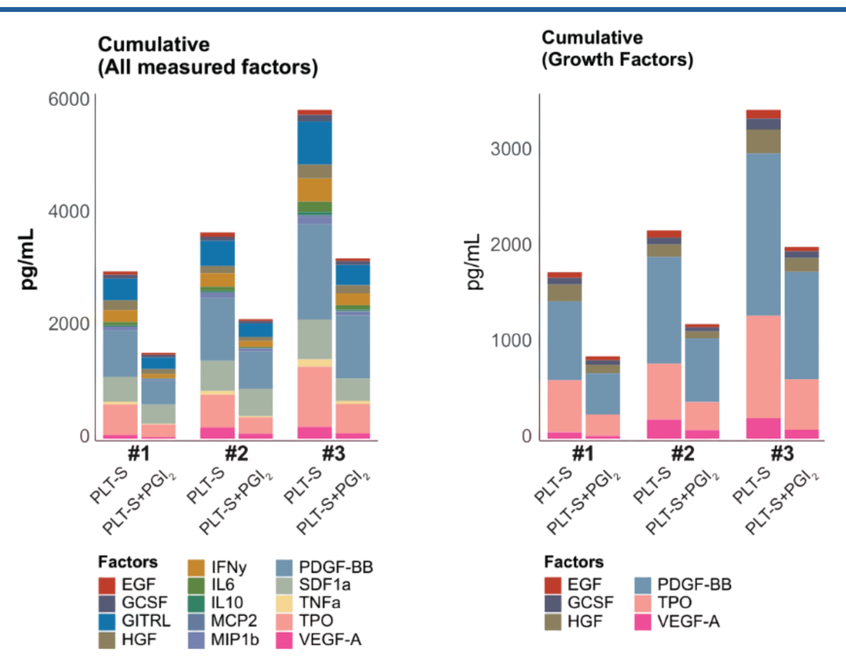
Platelet aggregation after TRAP-6 stimulation

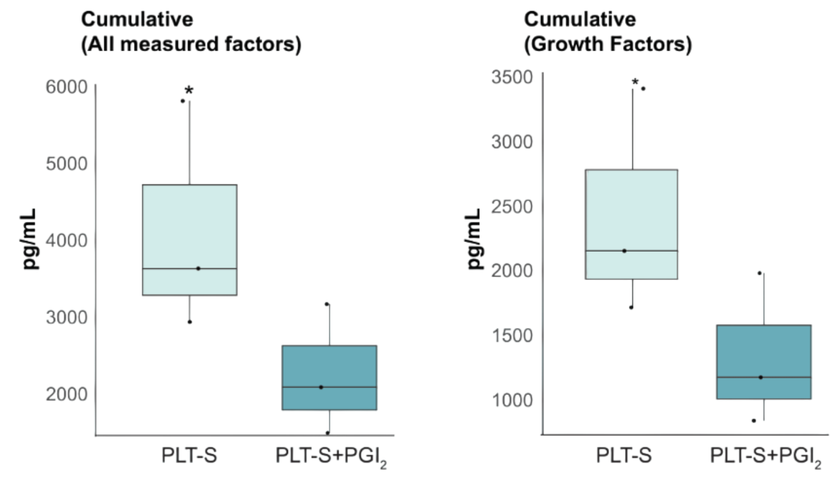
Representative cytospin images showing reduced aggregate formation in PLT-S+PGI_2 compared to PLT-S following TRAP-6 stimulation. Unstimulated controls show no aggregation.



Platelet degranulation assessed by CD62P expression

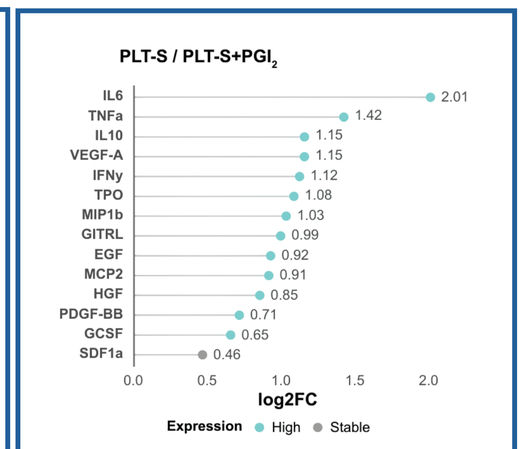
Flow cytometry analysis of CD62P (P-Selectin) membrane translocation after TRAP-6 stimulation. PGI_2 exposure significantly reduces CD62P surface expression.





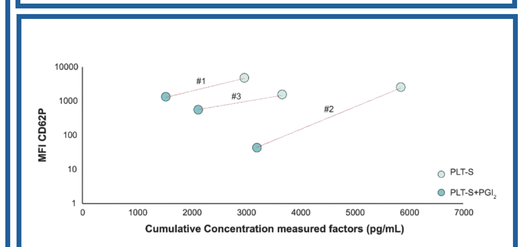
Total secreted bioactive factors

Cumulative concentration of measured analytes quantified by multiplex immunoassay. Secretomes generated without PGI_2 show significantly higher total bioactive content (mean ± SD; paired t-test, *$p < 0.05$).



PGI_2 alters global secretome molecular profile

The fold change (FC) between conditions was also analysed, revealing a downward molecular concentration trend in PLT-S+PGI_2 compared to PLT-S ($p = 0.05$).



Platelet degranulation correlates with platelet secretome composition

Positive association between CD62P expression (GeoMFI) and cumulative secreted factors. Reduced degranulation in PGI_2-treated platelets associates with diminished secretome enrichment.

Conclusions

PGI_2 exposure during platelet isolation compromises subsequent platelet activation and significantly alters the molecular composition of platelet secretomes. Omission of PGI_2 enables more efficient degranulation and generation of biologically enriched secretomes, supporting improved standardization and functional quality of platelet-derived bioproducts for translational applications.

Acknowledgement

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