Summer Studentship Project Report 2025

Title of Project
Investigating the Role of Oestrogen in Modulating Platelet Function in Women with HIV

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Lay Summary

Globally, over 35 million people are living with HIV, and more than half are women [1]. While antiretroviral therapy (ART) has transformed HIV into a chronic but manageable condition, it is well-known to be associated with an increased risk of CVD, likely driven by persistent immune activation, systemic inflammation, and off-target effects on platelet function. Additionally, these CVD risks are often worse in women with HIV than in men [2]. Despite this, women have been historically, and currently remain, underrepresented in HIV research [3].

Platelets are circulating cell fragments that play a central role in haemostasis and thrombosis. Their activity is thought to be influenced by hormones such as oestrogen, which modulate vascular tone, endothelial function, and platelet activation [4]. Oestrogen levels decline markedly during menopause; thus, post-menopausal women with HIV potentially experience compounding cardiovascular risk through reduced hormonal protection and enhanced inflammatory signalling. However, existing research into the effects of oestrogen on platelet function remains limited and often contradictory, with studies reporting both pro- and anti-thrombotic outcomes [5][6]. These inconsistencies highlight a critical knowledge gap in understanding how oestrogen signalling affects platelet activity.

Therefore, to investigate the role of oestrogen in platelet activation, specifically through the G protein-coupled oestrogen receptor (GPER) signalling pathway, the study examined how 17β-oestradiol (E2) influences platelet aggregation and how these effects are modulated by the GPER antagonist, G-15. By comparing platelet responses to E2 alone and in combination with G-15, the study aimed to distinguish receptor-mediated oestrogen effects from baseline platelet activity. Understanding how oestrogen signalling influences platelet activation in women with HIV may help explain their heightened cardiovascular risk and inform the development of more targeted therapeutic strategies.

Project aim

The overall aim of this project was to explore the effects of oestrogen on platelet function within the broader context of cardiovascular risk in women with HIV. By focusing on the signalling pathways through which oestrogen modulates platelet activation, the project aimed to provide new insight into sex-specific mechanisms underlying cardiovascular risk and thus support the development of more tailored approaches to managing health in women living with HIV.

Methods

Platelet Preparation and Aggregation Measurement
Platelet-rich plasma (PRP) was prepared from healthy donor blood samples and used to assess platelet aggregation. Aggregation was measured using a 96-well plate reader by recording changes in optical density following stimulation with the thrombin receptor-activating peptide, TRAP-6. Percentage aggregation was calculated by normalising the change in absorbance to platelet-poor plasma (PPP) and PRP controls in the absence of agonist.

TRAP-6 Dose-Response Optimisation
To validate the sensitivity and reproducibility of the assay, a dose-response curve was first generated using TRAP-6 at concentrations of 0.1, 0.3, 1, 3, 10, and 30 μM. This established a dynamic range of platelet aggregation responses and confirmed that the assay could detect graded platelet activation.

Assessment of GPER Blockade Using G-15
PRP was incubated with G-15 at concentrations of 0.1, 1, and 10 μM for 20 minutes prior to stimulation with TRAP-6. This experiment aimed to determine whether inhibition of GPER influenced platelet aggregation, providing insight into potential effects of endogenous oestrogen present in plasma and their potential mechanistic pathway.

E2 Treatment and G-15 Co-incubation
PRP was then treated with 17β-oestradiol (E2) at 0.04, 0.4, and 4 μM for 20 minutes before TRAP-6 stimulation. To assess receptor-specific effects, half of the sample were co-incubated with 0.1 μM G-15. The percentage aggregation was compared between E2-treated, E2 + G-15-treated, and vehicle (PBS) control samples to investigate the effects of oestrogen signalling through GPER on platelet aggregation.

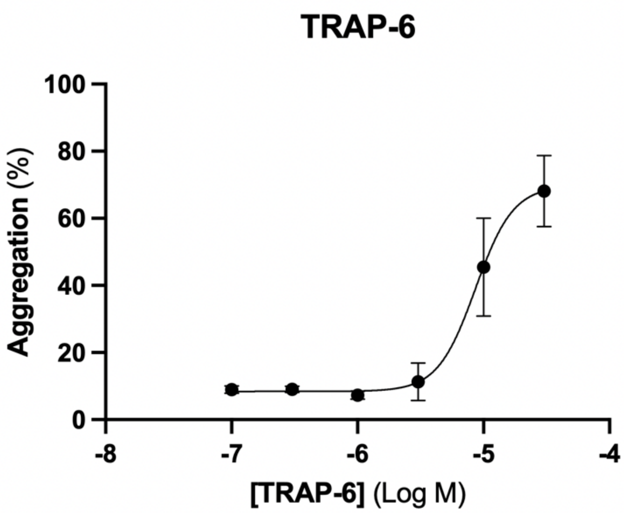
Flow Cytometry Analysis of Platelet Activation Markers
To complement the aggregation data, flow cytometry was used to measure specific markers of platelet activation in the same treatment conditions used for the E2 and G-15 aggregation assays, with G-15 applied at 1 μM. This enabled quantification of surface markers that reflect distinct stages of platelet activation, providing a more detailed analysis of functional changes induced by oestrogen signalling.

The following fluorescently labelled antibodies were used:

- PAC-1 (1:10 dilution): Detects the activated conformation of integrin αIIbβ3, indicating fibrinogen-binding capacity and late-stage platelet activation [7].
- CD107a (1:100 dilution): A marker of α-granule membrane exposure, reflecting degranulation during platelet activation [8].
- P-selectin (CD62P, 1:70 dilution): Identifies α-granule release and platelet–leukocyte interaction potential, representing an established marker of pro-inflammatory capacity [9].

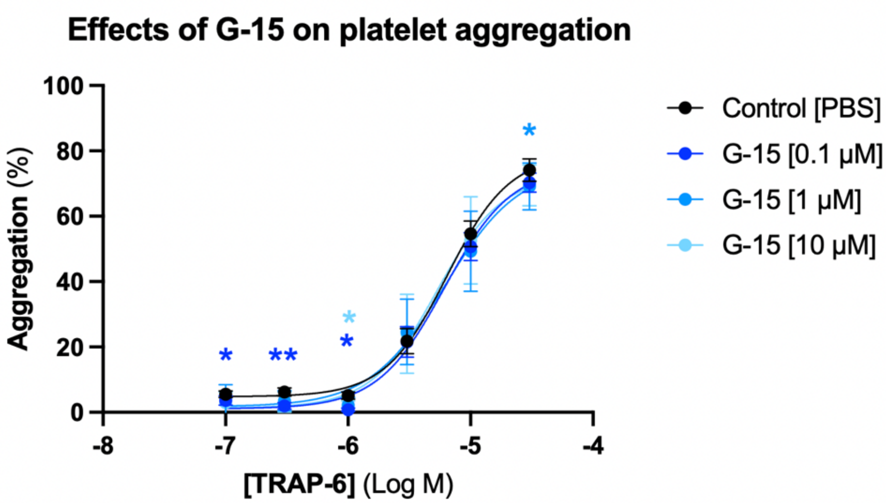
Mean fluorescence intensity (MFI) was used as a quantitative measure of surface marker expression, allowing direct comparison of activation levels across treatments. This method provided complementary evidence for oestrogen and GPER-mediated modulation of platelet activation beyond bulk aggregation measurements, which may not capture mechanistic intricacies.

Results



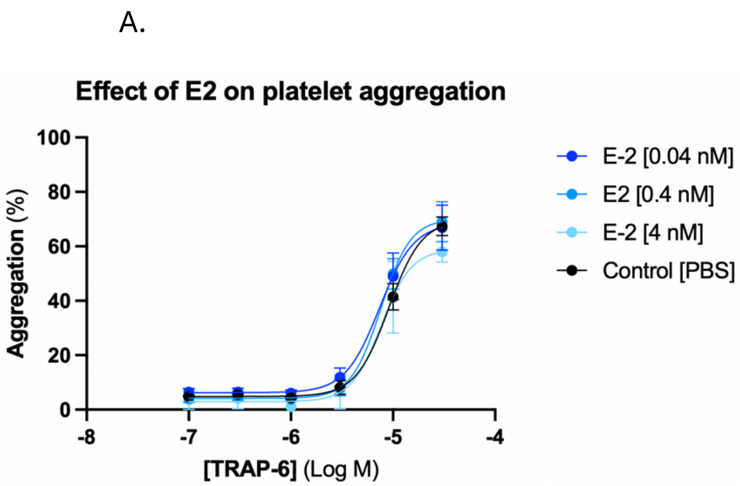
Initial optimisation using TRAP-6 demonstrated a clear concentration-dependent increase in platelet aggregation. The response validated the assay's sensitivity and established suitable conditions for subsequent experiments.

Figure 1. TRAP-6 dose-response curve for platelet aggregation.
PRP was stimulated with increasing concentrations of TRAP-6 (0.1–30 μM), and aggregation was measured by change in optical density using a plate reader. Data are expressed as mean ± SEM (n = 5).



Incubation with G-15 produced a small but statistically significant reduction in aggregation at lower TRAP-6 concentrations compared with PBS controls. However, no concentration-dependent effect of G-15 was observed, and aggregation responses converged at higher agonist concentrations. These findings suggest that G-15 could exert a slight inhibitory effect on platelet activation, potentially reflecting pro-thrombotic effects of endogenous oestrogen.

Figure 2. Effect of G-15 on TRAP-6–induced platelet aggregation.
PRP was pre-incubated for 20 minutes with G-15 at 0.1, 1, and 10 μM prior to stimulation with TRAP-6 (0.1–30 μM). Data are shown as mean % aggregation ± SEM (n = 5). Asterisks indicate significant differences compared with PBS-treated controls (*p < 0.05, **p < 0.01).



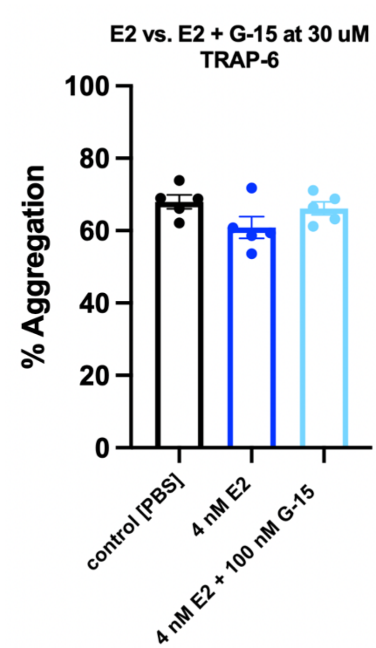
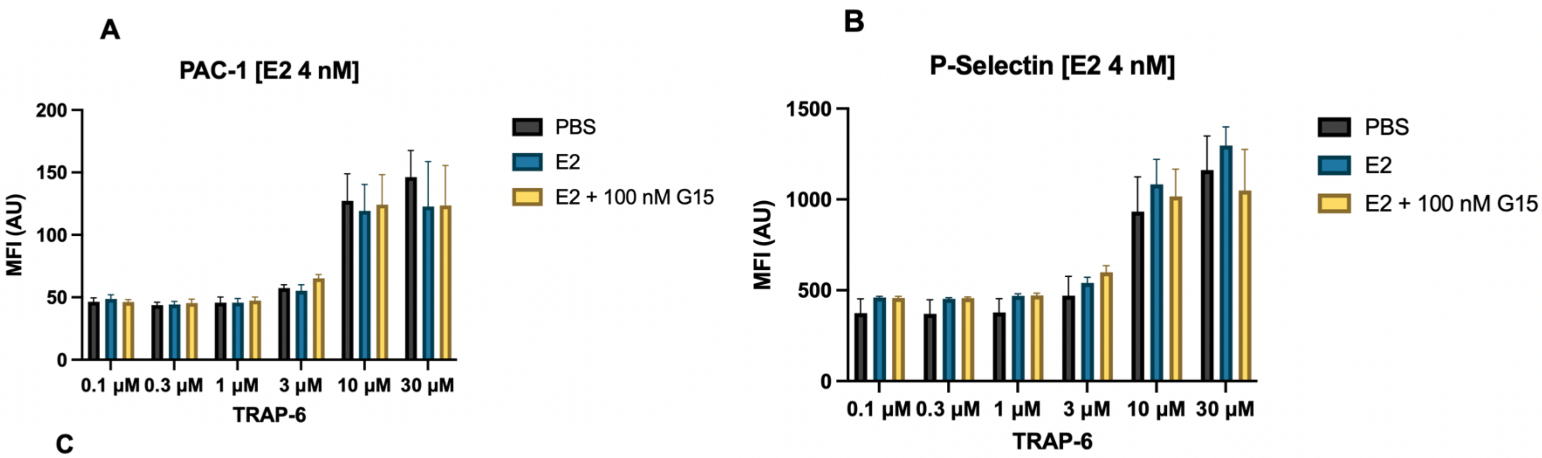


Figure 3. Effect of oestradiol (E2) and G-15 on platelet aggregation.
(A) PRP was incubated with increasing concentrations of E2 (0.04, 0.4, and 4 uM) prior to stimulation with TRAP-6 (0.1–30 μM). Data are shown as mean % aggregation ± SEM (n = 5). No statistically significant differences were observed across the E2 concentrations.
(B) PRP was treated with 4 nM E2 alone or in combination with 100 nM G-15 prior to stimulation with 30 μM TRAP-6.

Figure 3A shows a general trend in which treatment with E2 appeared to slightly reduce platelet aggregation while Figure 3B shows how co-incubation with G-15 may have partially reversed this effect. However, these changes were not statistically significant, and considerable variation was seen between biological replicates. As a result, no definitive conclusions can be drawn regarding the effect of E2 or G-15 on platelet aggregation.



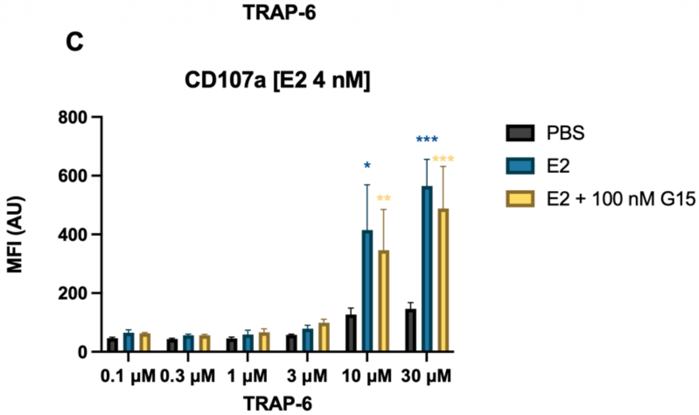


Figure 4. Flow cytometric analysis of platelet activation markers following E2 and G-15 treatment.

PRP was treated with 4 nM E2 alone or in combination with 100 nM G-15 and stimulated with increasing concentrations of TRAP-6 (0.1–30 μM). MFI was used as a quantitative indicator of marker expression. Data represent mean ± SEM (n = 5).

(A) PAC-1 binding increased with TRAP-6 stimulation and showed no significant modulation by E2 or G-15.

(B) P-selectin (CD62P) expression also rose in response to TRAP-6 but was not significantly affected by E2 or G-15 co-treatment.

(C) CD107a expression increased with TRAP-6 concentration, showing significant upregulation compared with PBS controls at 10 μM and 30 μM TRAP-6 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Treatment with E2 or E2 + G-15 did not significantly alter CD107a expression relative to each other.

Flow cytometry revealed that treatment with E2 seemed to significantly increase CD107a expression compared to PBS controls at 10 μM and 30 μM TRAP-6 stimulation, indicating enhanced platelet degranulation. Co-incubation with G-15 produced a slight reduction in CD107a expression relative to E2 alone, although this difference was not statistically significant. This trend suggests that GPER signalling may contribute to the oestrogen-induced increase in CD107a expression, but no definitive conclusion can be made. In contrast, P-selectin and PAC-1 expression remained largely unchanged across treatments both treatments, implying that oestrogen's effect may preferentially modulate secretory granule exocytosis rather than α-granule release or integrin activation.

Conclusion

This study utilised platelet aggregation assays and flow cytometry to explore oestrogen's effects on platelet activation. G-15 produced minimal changes in aggregation, while E2 and E2 + G-15 treatments showed variable, non-significant effects. Flow cytometry revealed that E2 markedly increased CD107a expression, partially reduced by addition of G-15, suggesting oestrogen may exhibit pro-thrombotic effects through enhanced platelet degranulation via GPER-linked mechanisms. Considerable variability between samples reflected challenges in controlling oestrogen-related factors such as endogenous hormone levels, donor menstrual cycle stage, etc. Further work with larger sample sizes and more focused cohorts (e.g. post-menopausal women) is needed to confirm these trends and define how oestrogen signalling contributes to platelet function in women with HIV.

What I gained from this project:
Throughout this project, I developed a range of valuable technical and professional skills. I gained hands-on experience in platelet biology, including preparing PRP from blood samples, performing aggregation assays, and using flow cytometry. I also had the opportunity to shadow other research groups in the department, which gave me exposure to a broader range of techniques such as PCR, Western blotting, gene transfection, and tissue culture. Professionally, I developed my skills in planning experiments, troubleshooting, and interpreting data, as well as communicating my findings effectively and working independently within a dynamic research environment.

How this project has influenced my long-term goals:
This project has consolidated my goal of pursuing a career in research and academia. In addition to developing my laboratory skills, I had the opportunity to assist my supervisor in generating MMI data interpretation questions for the upcoming cohort of medical students, which gave me valuable insight into the teaching and mentoring aspects of an academic career. The clinical relevance of my project has also strengthened my aspiration to pursue medicine alongside research, allowing me to contribute to translational science that bridges laboratory findings with patient care. Working on platelet biology in the context of women's cardiovascular health has deepened my passion for

understanding sex-specific disease mechanisms and reinforced my interest in women's health, particularly within cardiovascular science.

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