THE PLATELET SOCIETY

**Summer Student Project Report**

**Student**

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**Title of Project**

Platelet biology at fridge temperatures

**Supervisors**

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**Lay Summary**

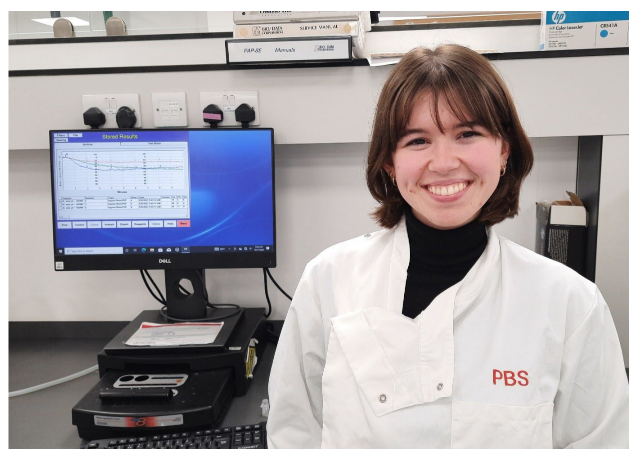
Platelets are used in transfusion medicine to stem bleeding in patients experiencing major blood loss[1]. Currently, platelets are stored at room temperature for only up to 5 days due to an increased risk of bacterial contamination with prolonged storage[2]. Recently, there has been an interest in the possibility of storing platelets at refrigeration temperatures to prolong storage time. However, it is unclear if cold temperatures are tolerated well by platelets. In this project we observed the effects on the structure and functionality of platelets when stored at refrigeration (4°C) or room (22°C) temperatures for a short time. To do this we used microscopy, biochemical assays, and platelet aggregometry. We found that rewarming platelets after a period of cold storage may, to some extent, reverse structural damage caused by cold storage. The aggregometry results did not show the effect we had expected based on earlier published reports, therefore further experimentation is necessary. In conclusion, I have learned how to work as part of team and perform different biochemical assays and how to prepare slides for microscopy.

**What was the aim of your project?**

The aim of my summer research project was to identify the changes that platelets undergo both structurally and functionally when stored at refrigeration (4°C) vs room (22°C) temperatures for several hours.

**How did you address the aim?**

Blood was obtained from healthy donors (Ethics approval 1504). Washed platelets were prepared by separating the platelet rich plasma from the blood sample and by two rounds of centrifugation to obtain a platelet pellet. Resuspended resting platelets were then either stored at room temperature (RT; 22°C) or in a cold room at 4°C. Samples were taken in the beginning (R1), after 2h at RT (R2), after 2 hours at 4°C (R3), or after rewarming to RT after 2h45min (R4) (see Figure 1A).




For STED microscopy, platelets were fixed with 4% paraformaldehyde and spun down onto fibrinogen-coated coverslips. Then, through several steps we added primary and secondary antibodies that would help to identify GPIb or beta-tubulin to visualise any GPIb

clustering or the unwinding of the microtubule marginal band using STED microscopy.

Alternatively, we used an ultracentrifuge and subsequent western blotting as a method to determine the ratio between soluble and polymerized tubulin to directly measure the depolymerization of microtubules.

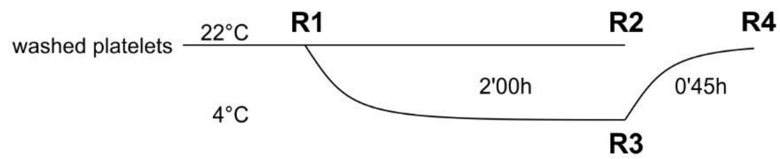
To test GPIb's ability to bind von Willebrand Factor (vWF) and agglutinate platelets, we added 1.5 mg/mL ristocetin and performed light transmission aggregometry by injecting 10, 20 or 40 µg/mL (final concentration) vWF to induce agglutination.

**What did you find out?**

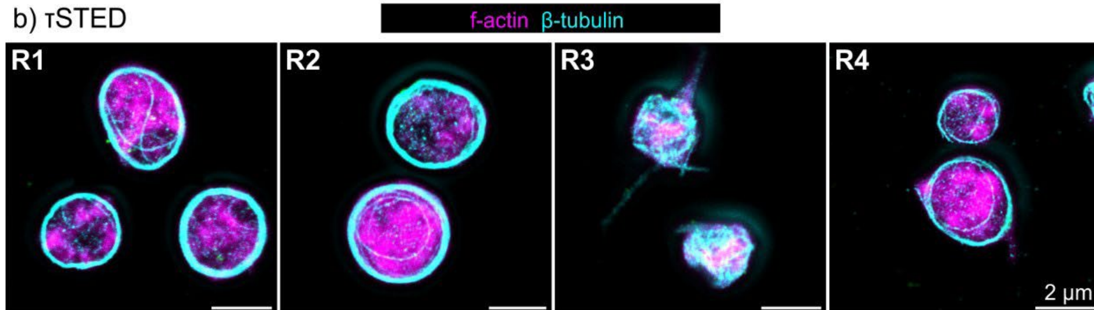
Using STED microscopy, we were able to see that when platelets were stored in cold temperature for 2 hours and then moved to room temperature for 45 minutes the marginal band disassembled slightly (Figure 1B). However, when platelets were stored in cold temperature only, the marginal band seemed to disassemble to a larger extent. Using the ratio between the tubulin staining intensity of an inner region (which normally is devoid of microtubules) and the outer region (which normally contains the marginal band) we saw that these changes were statistically highly significant (Figure 1C; one-way ANOVA with Bonferroni). This confirmed that cold storage does cause structural changes in the platelet, however, re-warming the platelets after cold storage may reverse this to some extent. The outcome of our western blots showed depolarisation of R3 and R4 as compared to R2 (Figure 1d). Interestingly, acetylation which is marker for stability is maintained in R1 and R2 but is reduced in R3 and R4 (Figure 1e). This result suggests that the depolymerisation of microtubules and their acetylation state might not recover from cold storage, in contrast to the organisation of microtubules as seen in the STED microscopy experiment. We have started to look at tubulin (de)acetylation to understand if this process could be targeted by deacetylase inhibitors.

Figure 2b shows LTA traces of R1 samples with varying amounts of vWF injected at time zero. We can discern that the agglutination of platelets is dependent on the concentration of vWF, and this dose dependent behaviour was confirmed by further experimental replicates (Figure 2c). Since cold-storage of platelets has been reported to induce GPIb clustering,3 which was concomitant to increased vWF binding interactions,4 we hypothesised that cold-stored platelets would show an increased agglutination response to low concentrations of vWF. However, R1 and the cold-stored sample R4 had a similar pattern of results, whereas the RT-stored sample R2 developed a higher response to the lowest concentration of vWF. Based on these outcomes, refrigeration with subsequent rewarming of the platelets before the addition of vWF did not seem to lead to increased platelet-vWF interactions. Further experiments are required to understand if GPIb clustering was happening upon cooling and/or if it was reversed upon rewarming.

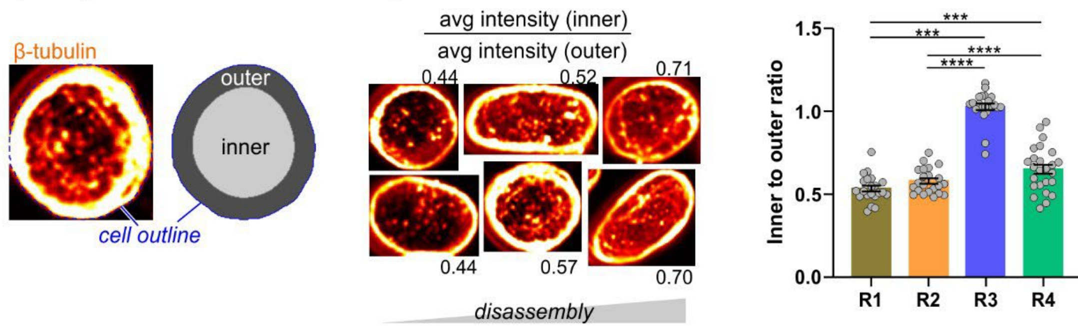
a) Temperature protocol



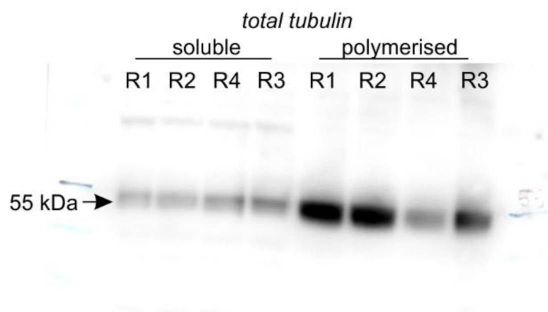

b) τSTED




c) Marginal MT band disassembly




d) Tubulin partitioning assay




e) Tubulin acetylation

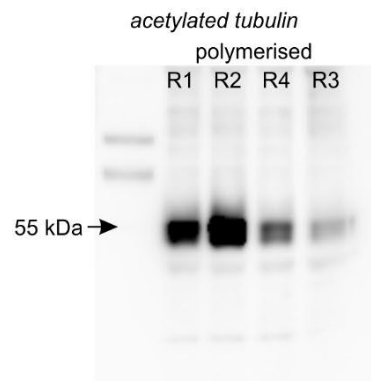
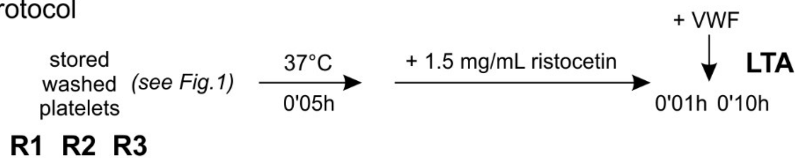
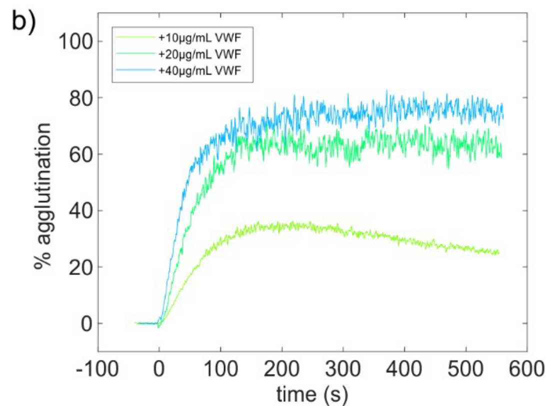



Figure 1.



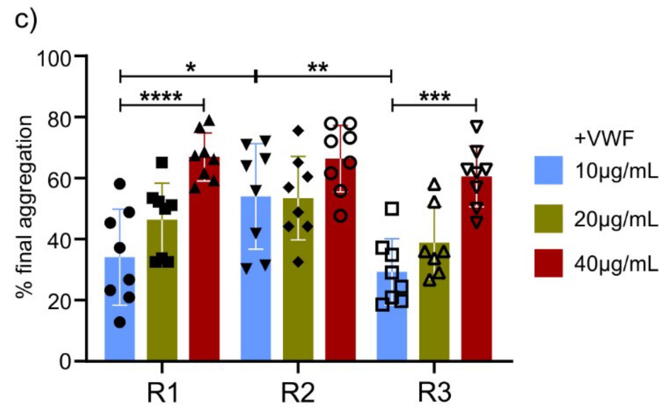




Figure 2

**What did you learn from participating in this project?**

Over the course of this project, I learned how to pipette, centrifuge, prepare chemical and blood component solutions and how to follow a protocol. These skills allowed me to independently prepare slides for microscopy as well as prepare western blots. Both methods have time sensitive components thus highlighting how important it was to schedule your week so that each experiment could be completed properly. Learning how to efficiently read research papers also proved beneficial to me in understanding how to identify and interpret key information. I found using STED microscopy to be the most interesting part of the project as platelets are very complex cells. It was fascinating to see how the temperature changes or possible human error can change their structure and functionality.

Having great, enthusiastic educators makes all the difference when learning new skills. The post-doctorate students and the main supervisor of the project were terrific at teaching and ensuring that I was understanding what was going on. Being included in the weekly meetings allowed me to observe and learn how to present your newest findings as well as recap what we had done during that week. Overall, this was an invaluable experience for me, and I will be able to carry over many of the things I have learned into my future endeavours.

**How has this project affected your long-term goals?**

This project was a wonderful experience and has spiked my interest in a variety of different medical specialties that I may not have considered prior to participating in this project. I am thrilled to have had this opportunity and look forward to my medical rotations and electives where I will get to see how platelet research and haematology can benefit patients. In the short term, I am excited to continue this project in my 6-week dedicated research period this autumn semester! Proceeding with this project allows me to gain an even

greater understanding of platelets, platelet transfusion and haemostasis. I look forward to this period and the opportunity to continue my work with such a great research team.

**References**

1. McCullough, J. (2010). "Overview of Platelet Transfusion."
2. Kaufman, R. M., et al. (2015). "Platelet Transfusion: A Clinical Practice Guideline From the AABB."
3. Hoffmeister, K. M. (2003). "The Clearance Mechanism of Chilled Blood Platelets." Cell.
4. Chen, W., et al. (2017). "Refrigeration-Induced Binding of von Willebrand Factor Facilitates Fast Clearance of Refrigerated Platelets."