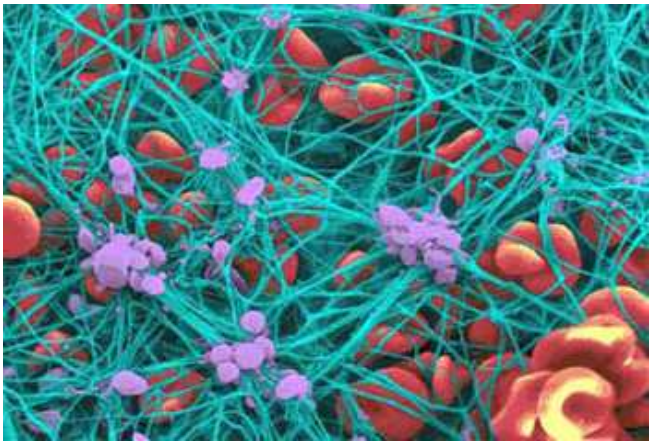
Platelets - Biology and Assessment of Suitability for Transfusion

Platelets are the key particles which drive haemostasis and coagulation in the body. Thus they are very sensitive to physiological insults, which creates problems upon their collection for transfusion to thrombocytopenic patients, as it is more advantageous to prevent their activation, or the platelet storage lesion, for as long as possible. The longer they are stored for, the more advanced the lesion progresses, and so suitable tests to assess viability for transfusion are performed. However it is more important that novel techniques to hold the platelet storage lesion at bay are developed, to retain stores of platelets and to prevent shortages of these life-saving bodies.

The separating tissue between the outer environment and a person's peripheral blood vessel may only be a few millimetres, or less, at certain locations in the body. Should this barrier be breached, the person could potentially haemorrhage to the point of fatality, if the coagulation system was not present, and the blood platelet is the key to this system. However, in certain circumstances, such as in thrombocytopenia, the system is compromised by a lack of functional platelets (PLTs), and in such cases, platelet transfusion may be the only life-saving treatment available. This dissertation aims to look at the physiological importance of platelets, how they are prepared for transfusion, and also the difficulties experienced in this preparation and how they are, and may potentially be in the future, overcome.

Platelets are anucleated cells/fragments, produced by megakaryocytes in the bone marrow, naturally having a discoid morphology with dimensions 3 μm x 0.5 μm, expressing ABO antigens, human leukocyte antigen-I and human platelet antigens.[1,2] The normal range for platelets in the blood is $150x10^9$-$400x10^9$/L, and each platelet survives in the blood for approximately 9.5 days.[3,4] The loss of endothelial integrity in a blood vessel exposes the collagen in the subendothelium, which expresses a ligand called the von Willebrand factor (vWF). A receptor complex for vWF is present on platelet membranes, and is made from glycoprotein-Ib (GPIb), GPV and GPIX, anchoring the platelet to the subendothelium. This primary step in the haemostatic response is secured by further adhesion molecules, e.g. GPVI binds fibrillar collagen and $\alpha_6\beta_1$ integrin binds laminin, and signalling occurs inside the platelet.[3,5]

Src kinases and phosphatidylinositol 3-kinase, PI(3)K, transduce the signal, activating phospholipase C-γ to produce inositol trisphosphate, IP_3. This complexes with its receptor, IP_3R, on the dense tubular system (DTS) inducing Ca^{2+} release. Following this, many Ca^{2+} dependent activities occur, such as thromboxane A_2, TXA_2, (produced from prostaglandin H_2) and ADP production and release. These act in paracrine and autocrine fashions on platelets, increasing adhesion molecule affinities for subendothelial matrix proteins, thus further securing the adhesion. Also TXA_2, ADP, thrombin and the other released factors typically act through G protein coupled receptors (GPCRs). For TXA_2 and ADP, the Gα subunit activates PLCβ to increase intracellular Ca^{2+} from the DTS even more, using IP_3, and also activates PI(3)K to activate the Rap1b GTPase, respectively. Therefore TXA_2 augments the exocytotic release of granules further, and ADP activates talin, via

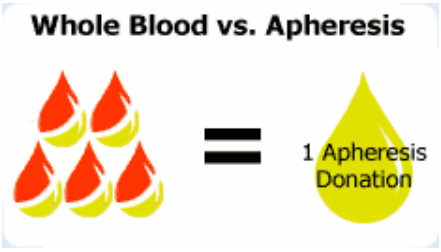


Rap1b, to induce actin modification. This cortical remodelling cause pseudopod formation, and increased adhesion.[6,7]

Artificially coloured scanning electron micrograph of a whole blood clot.[16]

Intracellular Ca^{2+} also causes the expression of CD62 (P-selectin) to form aggregates of platelets. These aggregates, again by Ca^{2+} signalling, express outer membrane phosphatidylserine, a classical apoptotic signal. In this circumstance it is also a coagulation signal, as it creates an unusually strong negative outer membranous charge, attracting coagulation factors such as factors Va and Xa to form thrombin from prothrombin. This thrombin formation through the aggregate further increases the activation already discussed, but also cleaves fibrinogen to form fibrin. This stabilises the aggregate to the status of a clot, which forms a plug once it retracts. During the course of this, microparticles (40nm x 1µm) are also released as exocytotic buds from the platelets. These are part of the apoptotic process, but are capable of the functions outlined above, and thus contribute to the role of platelets in haemostasis.[3,6,7]

There are a few methods by which these crucial particles are obtained from donors to be prepared for transfusion into cancer or thrombocytopenic patients. The standard whole blood derived platelet rich plasma (WBD-PRP) comes from normal donations. A closed system donation of the 450ml unit is received, and is soft spun producing a platelet concentrate in the supernatant. This supernatant is then hard spun to produce a platelet sediment, and approximately 4-8 of these are combined to give 1 platelet unit. This is a comparatively cheaper method to use, but exposes the recipient to an increased number of donors, thus presenting more potential for transfusion reactions (TRs) or infections. A Buffy coat can be used, which uses the same method as WBD-PRP but exchanges the spin order i.e. hard spin first, soft spin second, and finally apheresis can be used. Apheresis is the most expensive procedure, and reduces the amount of blood components received, this also reduces the number of donors as well as the risk of TRs or infections.[1,8] It is important to have leukoreduced units of platelets as nearly 90% of TRs are cytokine induced, and 10% by antibodies. Thus, the TRAP Study Group strongly encourage the treatment of units with UV_B light, or amotosalen, to reduce the number of active leukocytes present.[9,10]



Ratio of WBD donors vs.Apheresis donors with respect to platelets recovered[17]

In addition to this, platelet function must be maintained without contamination, so the polyolefin bags used (such as PO-80), must have a large surface area, be thin and of an adequate material for O_2/CO_2 exchange to promote aerobic metabolism and ATP maintenance. A drop in ATP concentration by 30%, or to below 4 µmol/10^{11} PLT can be deemed notably detrimental to function and morphology and this can be detected by pH testing.[9] Anaerobic respiration will cause a rapid drop in glucose and a rise in lactate which displaces bicarbonate, thus reducing the pH. So a range of pH6.2-7.4 has been generally attributed as satisfactory.[1,8,9,11,12,13] To enable good O_2 access, and avoid sedimentation, oscillating agitation must be maintained at 22°C,[12] and due to potential bacterial survival and growth at this temperature, a shelf life of 5 days for non-bacterial culture tested samples has been set.[1,3,4,6,14]

Platelets are made in the body, and are designed to resist any insult to the body's integrity, however the removal of anything from the body itself is an insult. So from the point of donation platelets experience some degree of activation, and for the past 20 years and still today efforts are made to resist this activation, also known as the platelet storage lesion (PSL). Shrivastava describes the PSL rather exhaustively as *"the sum of all deleterious changes leading to progressive damage in platelet structure and function that arise from the*

time blood is drawn from a donor to the t ime platelets are transfused to a recipient." [4] As can be surmised from this statement, the PSL refers to morphological, metabolic and apoptotic changes, all affecting the haemostatic function, the activation status and activation potential of the platelets. This starts and progresses from the point of donation, thus fresher platelets are better for transfusions. [3,4,6,9,11]

Generally, the PSL consists of morphological changes and a reduction in the platelets' physiological responsiveness to typical agonists. The most commonly utilised change is the morphological change from smooth discoid platelets, to spiny spheres potentially extending pseudopodia and releasing microparticles. This is due to actin and microtubule remodelling, caused by Ca^{2+} release from the DTS by mechanisms previously described, and thus highlights the activated status in the PSL. This can be affected by the type of collection, with apheresis promoting less of a PSL than WBD -PRP or Buffy coats, due to the amount of shear stress imposed on the platelets. [3,4,9] Following collection, oscillating agitation should be adopted, as this reduces the amount of foreign surface contact the platelets experience with the container, thus reducing their activation. [12] The fact that the platelets are activated reduces their potential for further activation in the recipient, thus the PSL is characterised by a rise in activation, and a reduction in future activation potential.

Not a vast amount is known about the PSL at a biochemical level but a few major aspects have been elucidated. A seemingly common occurrence in the PSL is an alteration to the vWF binding complex (GPIb/GPV/GPIX). This reduces the platelets ability to adhere to vWF, and thus the potential for further activation and aggregation. This could be due to metalloproteinase release by the platelet in response to shear stress, causing GPIbα cleavage. The complex also commonly clusters, creating a ligand for the phagocytosis -inducing αMβ2 receptor on macrophages. This provides an additional 'apoptotic' signal for the recipient's Küppfer cells to use to target the platelets, along with the CD62 and the elevated phosphatidylserine, to remove them from the circulation. This is particularly heightened in cold storage, e.g. at 4 °C, leading to 27% reduction in platelet recovery and circulatory survival after 72 hours storage, highlighting why this is not a viable storage condition. [4] The platelets are also characteristically desensitised to agonistic stimulation. This can be caused by $P2Y_1$ and $P2Y_{12}$, and $P2X_1$ receptor desensitisation to ADP and ATP respectively, [6] and the production of calpal in containing microvesicles. This compound degrades actin, talin, tubulin, and vinculin reducing the platelet's ability to change morphology, thus removing its response to pro -coagulant agonists. [4]

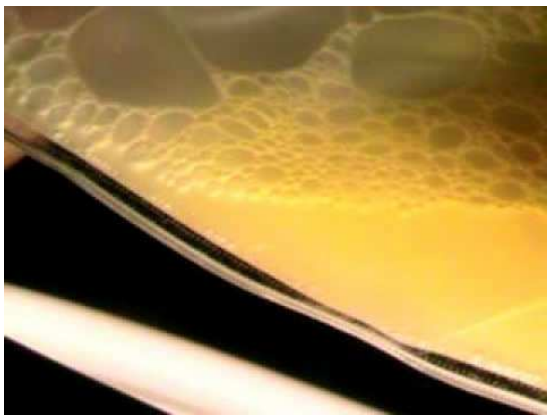


Image of platelet swirling. The scattering of light photons creates a non uniform presentation of the light passing through the discoid platelets in the sample [18]

It is important to discount platelets displaying advanced PSL for transfusion. There is no set automated test as yet, but a few standard ones that are employed. There are 2 that are focussed on in a clinical setting, and these are swirling and pH testing. Both are simple to perform, accurate but indirect assessments. Discoid platelets have a non-uniform morphology, thus when they move in agitation, they can scatter or diffract light. PSL platelets have pseudopodia and/or are spherical, which removes this ability. Thus when agitated, the display of 'swirling' provides an indirect indication of good quality platelets. [1,3,12] If a pH value outside the range of pH6.2 -7.4 is detected, this is evidence enough of a high lactate

concentration and metabolically unviable platelets.[1,6]

More complex tests can be performed, but these are typically reserved for the research setting. Flow cytometric analysis of microparticle presence, aggregation and surface marker presence such as GPIb or phosphatidylserine (using Annexin V) has been used in some studies,[4,6,15] or light scattering ability. This however assesses uniformity in static light scattering, as in normal flow cytometry, without marker analysis. Dynamic light scattering is much more useful, as instead of the target platelet being in a 'fixed focus point,' they move. More fluctuations in light intensity highlights more diverse contours on the membrane, and so a discoid morphology. This applies the same theory as the swirling test.[6]

'Pre' and 'post' treatment comparisons have been used also, such as treating a sample with an agonist such as ADP, or EDTA (normally an anticoagulant but as it is a Ca^{2+} chelator it induces apoptotic and pro-coagulation actions[11]) to cause changes such as in mean platelet volume (MPV). A major difference between non-EDTA MPV and EDTA-treated MPV reveals high activation potential, and thus transfusion viability.[6,7,11,12,15] Hypotonic shock response has also been used in this way.[6,9] These all focus on the PSL, however the shelf life of platelet is determined by the potential of bacterial contamination. Testing for bacteria could potentially extend the limit from 5 days to 7 or 9 days, if a negative result is presented. As some of the more common infections, such as *S. epidermidis*, have slow incubation times, a later test would be required, but a conclusive test at day 4 would reveal 97.9% potential infections. So, if a slow, yet accurate test was started at day 1 or 2 to be concluded at day 3 or 4, shelf life could be justifiably extended, making the PSL the length determining factor, not the possibility of bacterial contamination.[14]

Should the above proposal of early bacterial and viral testing be producible, the focus then would be the inhibition or prolonging of the PSL. Addition of inhibitors such as prostacyclin to stop ADP release, cytochalasin B to prevent actin remodelling, or metalloproteinase inhibitors to prevent the vWF receptor complexing are viable suggestions, however the issue of their removal, and platelet reactivation needs considered.[4,6] Metabolic arrest induced by anti-mycin A for 1 hour, then 4°C storage for 48 hours, followed by warming in a high glucose concentration medium has been proposed to correct this, but this highlights the requirement of a high level of familiarity and expertise required by technicians[6].

Platelets are invaluable in medicine, particularly for those undergoing treatment for cancers, and thrombocytopenia. However, their high value is equated with a high level of care and treatment to guarantee their integrity and viability for those patients who need them. The above suggestions reveal the requirement for novel, innovative, drastic techniques to help maintain the limited stock of platelets transfusion services possess for as long as possible, retaining sterility from microbes, including viruses, and their activation potential.

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