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A Systematic Comparison of Protocols for the Isolation of Platelets From Whole Blood For RNA Analyses

Introduction: Platelets whilst anucleate are now known both to contain transcriptional machinery, messenger RNAs and micro RNAs derived from their parent megakaryocytes, and to be transcriptionally active (1). This recent realisation has stimulated studies to characterise platelet messenger and micro RNAs. However the low abundance of RNA within individual platelets and the contamination of samples with other cell types, in particular white blood cells (WBCs), present major complications for downstream RNA applications. Because of this there are many diverse protocols for the isolation of platelets from whole blood but currently no consensus as to which is best. We therefore aimed to compare a range of platelet isolation protocols for their abilities to remove contaminating WBCs from platelet samples.

Methods: Blood was obtained by venepunture from healthy volunteers (study approved by NHS St. Thomas Hospital Research Ethics Committee; Ref 07/Q0702/24) into sodium citrate (3.2%) or heparin-coated vacutainers. Citrated blood was centrifuged (175 x *g*, 15 min) to obtain platelet rich plasma (PRP). PRP was incubated in the presence/absence of CD45 positive magnetic beads (MiltenyiBiotec) and run through magnetic activated cell sorting (MACS) columns. Washed platelets were generated from PRP by addition of prostacyclin (2 µg/ml) and apyrase (0.02 U/ml), centrifugation (2300 x *g*, 10 min) and resuspension in a modified Tyrode's buffer. Platelet suspensions were then passed through a 5 µm syringe filter. All samples were incubated with anti-human CD45 PerCP-Cyanine5.5 (1:60) to identify WBCs, anti-human CD61 APC (1:25) to identify platelets, and CD62P (1:15) to determine activation (all eBioscience, UK).Flow cytometry was then used to quantify WBC contamination per 100,000 single platelet events or CD62P expression. Data were analysed using a paired two-way or one-way ANOVA. For qRT-PCR analysis, RNA was extracted from whole blood or PRP using PAXgene whole blood or RNeasy extraction kits (Quiagen, UK). cDNA was synthesized using the SuperScript III System (LifeTechnologies). qRT-PCR was performed using TaqMan probes for genes specific to either platelets (GP6, P2RY12, PF4) or WBCs (ANPEP, MPO, PTPRC) on the Applied Biosystems 7900HT System. Data was analysed using the formula 2^-(GEOMEAN(Platelet markers)-GEOMEAN(Leucocyte markers)) and significance assessed using column statistics with a one sample t-test.

Results: In freshly collected blood there were 2263±161 (mean±s.e.m.) CD45-positive events per 100,000 platelets (n=8). All platelet isolation methods produced marked reductions in CD45-positive events: standard PRP, 14±4; washed platelets, 25±10; syringe filter, 21±5; MACS minus beads, 12±3; MACS plus beads, 20±6 (per 100,000 platelets). No significant differences were seen between the different isolation methods ($p>0.05$). In addition, qRT-PCR data showed that all platelet isolation protocols significantly increased the relative expression of platelet specific genes compared to whole blood. The greatest change occurred between whole blood and PRP with a 560-fold increase in platelet gene expression relative to WBC gene expression. Syringe filtering, but not the other methods, significantly increased markers of platelet activation.

Conclusion: Simple centrifugation of whole blood to obtain PRP is equally effective at reducing WBC contamination as other methods and is much less laborious and expensive. Importantly, simple centrifugation also removes the need for extensive sample manipulation that can activate platelets and so potentially modify platelet phenotypes and/or bias towards particular platelet subpopulations.

Reference:

(1) Rowley JW, Schwertz H, Weyrich AS (2012). Platelet mRNA: the meaning behind the message. Current Opinion Hematology, 19(5), 385-391