Separation of Platelets from Whole Blood

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Purpose

This protocol describes how to isolate human platelets from whole blood. Isolated platelets are used for static adhesion assays, for flow chamber assays, flow cytometry measurements, etc.

Materials

- Citrate buffer (25 g sodim citrate, 8 g citric acid, 500 ml H_2O_2).
- Prostaglandin E_1 (PGE_1).
- ACD buffer (6.25 g sodium citrate.2 H_2O , 3.1 g citric acid anhidrous, 3.4 g D-glucose in 250 ml H_2O).
- Hepes-Tyrode buffer pH 7.4 (134 mM sodium chloride, 12 mM sodium bicarbonate, 2.9 mM potasium chloride, 0.34 mM sodium phosphate monobasic, 1 mM magnesium chloride (avoid for adhesion assay), 5 mM Hepes, 5 mM glucose, 1 % BSA).

Procedure

1. Draw 45 ml blood into a syringe containing 5 ml citratebuffer. *Optional:* Add 2 micromolar PGE1 to avoid platelet activation.
2. Transfer to a 50 ml tube and centrifuge at 150g for 15 minutes at room temperature.
3. Carefully transfer the upper phase (Platelet Rich Plasma) to a 15 ml tube and add 1/10 volume of ACD anticoagulant.
4. Pellet platelets by centrifugation at 900 g for 5 minutes at room temperature. *Note:* After centrifugation, supernatant still contains a significant number of platelets and can be collected for experiments.
5. Resuspend the pellet in 5-10 ml Hepes-Tyrode buffer.

Keep platelets at room temperature throughout the experiment. Freshly isolated platelets should be used within 2 hours.