Supplementary Data

Supplementary Methods

Preparation of fresh and expired platelets

Peripheral blood from normal donors was collected into sodium citrate Vacutainer tubes (BD Biosciences) under a protocol approved by the Northwestern University Institutional Review Board. Within 30 min of collection, blood was centrifuged at 800 *g* for 5 min with no brake. The platelet-rich plasma was transferred to a polypropylene conical tube and an equal volume of HEP buffer (140 mM NaCl, 2.7 mM KCl, 3.8 mM HEPES, 5 mM EDTA, pH 7.4) was added. The tube was centrifuged at 100 *g* for 10 min to pellet any contaminating red and white blood cells. Approximately 2/3 of the platelet-rich plasma was centrifuged at 1000 *g* for 10 min to remove the platelet-poor plasma. One milliliter of modified HEPES/Tyrode's (HT) buffer (10 mM HEPES, 137 mM NaCl, 2.8 mM KCl, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.4 mM Na_2HPO_4, 0.35% BSA, 5.5 mM glucose, pH 7.4) was gently added twice to remove any remaining platelet-poor plasma on top of the platelet pellet. The platelet pellet was gently resuspended in HT buffer. Before analysis, $CaCl_2$ was added to a final concentration of 2 mM. Six-day-old, 200- to 300-mL apheresis platelet units, which had expired at midnight on the fifth day of storage, were obtained from the Northwestern Memorial Hospital Blood Bank. Platelets were isolated from the platelet-rich plasma as described above.