Supplementary Material to Zhang et al. “Contact- and agonist-regulated microvesiculation of human platelets” (Thromb Haemost 2013; 110.2)

SUPPL. METHODS

Confocal microscopy: PRP was incubated with fibrinogen immobilized to a culture dish for 30 min at 37°C, washed, and reviewed under a confocal microscope (Olympus IX81, Hicksville, NY) at room temperature without fixation. Time-lap images were acquired continuously for 30 min at a rate of 1 frame/min.

Scanning electronic microscopy: PRP was incubated with fibrinogen immobilized to carbon grids for 30 min at 37°C, fixed in 3% of glutaraldehyde, dehydrated with 70%, 90% and 100% of ethanol, and rehydrated with Tert-butyl alcohol. Platelets were then gold-coated and reviewed under a scanning electronic microscope (JSM-6380Lv, JEOL Ltd., Japan) at 30 kv.
Suppl. Figure 1: (A) A schematic diagram of the HPICM setup. HPICM controlled the vertical Z direction Piezo for hopping the nanopipette as well as the XY plane by two PIHera Piezo. When a probe approached to platelet, a reference DC current was measured as an average ion-current flowing into the nanopipette. A 0.4% reduction of such ion-current was set to maintain the separation between platelets and the nanopipette. (B) Flow cytometry detection of platelet microparticles defined as 50 nm – 1.0 µm particles that were stained positive for CD61 and bound annexin V.
Suppl. Figure 2: Platelet morphologies by AFM: PRP was incubated with immobilized fibrinogen for 30 min at room temperature and scanned at a rate of 0.3 Hz in a contact mode with DNP-S cantilevers (fo = 12-24 kHz, k=0.06 N/m, Bruker Corporation, Santa Barbara, CA). Platelets show morphologies indicative of fully spread and non-spreading before (A), but only spread morphologies after fixation with 10% formalin (B). A representative fully spread platelet is measured (C). When a cantilever was set based on the peripheral membrane, it sometimes scratched the pseudocenter region during scanning (D, bar = 5 μm).
Suppl. Figure 3: Images of platelets on fibrinogen: Platelets adherent to fibrinogen are either fully spread (LDSS, arrowhead) or non-spreading (HDBS, arrow) under confocal microscopy, similar to those observed with HPICM (A. bar = 30 μm). Both LDSS (asterisks) and HDBS (arrows) platelets were also observed under scanning electronic microscopy after fixation. LDSS platelets maintain contacts, whereas HDBS are most singlet platelets (B, x 5,000). Panel C shows a LDSS platelet (asterisk) contacts with a HDBS platelet, which appears to be in transition to LDSS morphology (x10,000).