Supplement Number 1

Microfluidic determination of lymphocyte vascular deformability: Effects of intracellular complexity and early immune activation

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Microfluidic Device Manufacturing: The mold for the microstructures consists of two photo-lithographically defined layers fabricated on a silicon wafer. The sorting region containing a matrix of funnels was fabricated using SU-8 3010 photoresist (MicroChem, Newton, MA, USA) at thickness of 11 μm. The supporting microfluidic channels were made from SU-8 3025 photoresist with a thickness of ~25 μm. The patterns for both regions were initially drawn using AutoCAD software, which was sent to Advanced Repro and CAD/Art Services for the fabrication of chrome mask (sorting region) and transparency mask (supporting channel region), respectively. These masks were used to create SU-8 photoresist features using mask aligner (CANON PLA-501F) in the cleanroom.

Photolithography: The SU-8 3010 (MicroChem, Newton, MA, USA) microstructures were fabricated on a cleaned 100 mm silicon wafer. After dehydration baking at 200°C for 5 minutes, photoresist SU-8 3010 was spread onto the wafer at 1500 rpm for 30 seconds to create a thickness of approximately 11 μm, as measured using a profilometer (Alpha step 200). The wafer was then soft baked at 95°C for 4 minutes before being exposed to UV light in a mask aligner with uniform exposure for 40 seconds. The exposed wafer was given a post exposure bake at 65°C for 1 minute, 95°C for 3 minutes and then 65°C for 1 minute. Finally, the wafer was developed using SU-8 developer (MicroChem). The geometry of the SU-8 3010 photoresist was stabilized by further baking with ramped temperature at the acceleration of 100 °C/hour from 40°C to 200°C, held at 200°C for one hour, and then

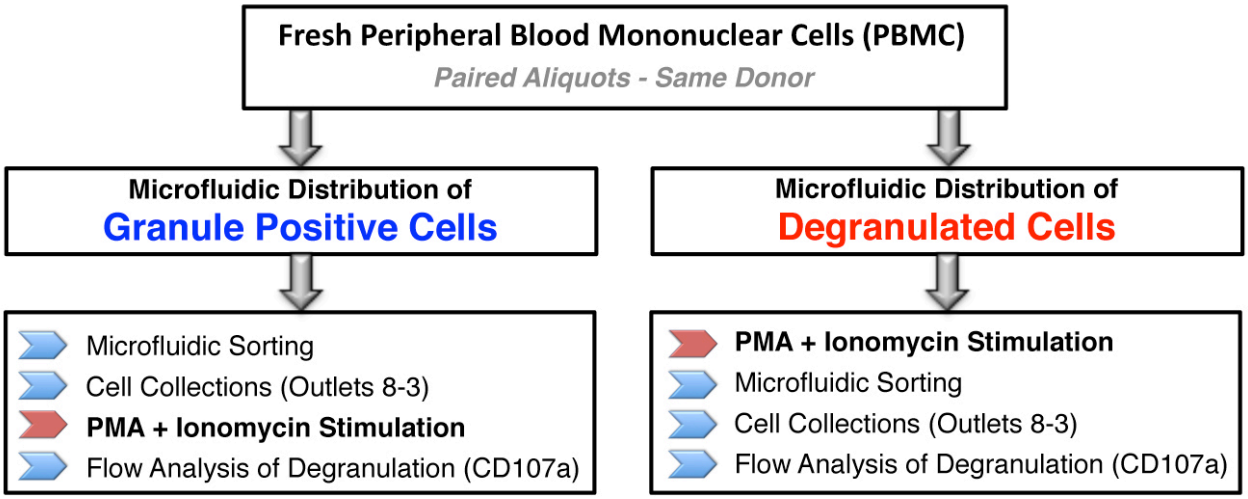
gradually cooled to 40°C. The SU-8 3025 microstructures were added to the SU-8 3010 silicon wafer via spin-coating (4500 rpm for 30 seconds). The coated wafer was soft baked at 65°C for 1 minute, 95°C for 4.5 minutes, and then 65°C for 1 minute. The designed transparency mask for the SU-8 3025 pattern was then aligned with the SU-8 3010 pattern and exposed for 55 seconds. After waiting for approximately 30 minutes, the wafer was developed using SU-8 developer (MicroChem). The finished structure was measured to be 18-20 μm in thickness.

Soft-lithography: Polyurethane-based plastic (Smooth-Cast ONYX SLOW, Smooth-On) was used to make replicas of the microstructures on the master silicon wafer, method described by Desai *et al.* [68] PDMS microfluidic devices were then fabricated from these molds using soft-lithography of RTV 615 PDMS (Momentive Performance Materials). After baking in 65° oven for ~3 hours, the cured microfluidic device was removed from its mold. Holes were punched using a 0.5 mm outer diameter hole punch (Technical Innovations, Angleton, TX, USA) in order to fit the fluidic introduction ports for cross flow, sample and oscillation inlets. The outlets were punched using a 3 or 4 mm diameter puncher. The microfluidic device was bonded by oxygen plasma (Model PDC-001, Harrick Plasma) to a layer of PDMS spin-coated onto a blank silicon wafer at 1500 rpm for 1 minute and then subsequently bonded to a standard microscope slide (50×75mm, Fisher Scientific).

Instrumentation and experimental set-up: As illustrated in **Fig. 1A**, the instrumental experimental platform is composed of three parts: pressure controllers; fluid reservoirs; and the core microfluidic ratchet device. Sample and medium fluids were contained in the

15 ml conical tubes (**Fig.1A(a-d)**) (Falcon, Fisher Scientific), sealed with custom-made caps. The pressurized reservoirs, via two pressure control systems, push fluid into the microfluidic device through 0.5 mm ID flexible Tygon tubing (Cole-Parmer) attached to 19 mm long stainless steel tubing (23 gauge; New England Small Tube, Litchfield, NH, USA) anchored to custom punched holes in the chip. Two pneumatic pressure control systems are used to pressurize the reservoirs to control fluid flow within the microfluidic channels. The first system is a custom pressure controller designed to supply pressure from 0 to 400 kPa bar using manual pressure regulators (Omega, McMaster-Carr). This is utilized to regulate the air pressure into reservoirs (c) and (d) to control upward and downward oscillatory flow. On/off pressure control is enabled using solenoid valves (Pneumadyne, McMaster-Carr) activated by MOSFET switches that are controlled using a MSP430 microcontroller (Texas Instruments) integrated on a printed circuit board. The MSP430 is controlled using a Visual Basic user interface program on a PC. The second pressure control system is the MFCS-4C system (Fluigent SA, Paris, France). This system supplies more precise pressure within a range of 0 to 100 kPa, with a resolution of 0.03 kPa. This is utilized to regulate the air pressure into reservoir (a) and (b) to control the cross flow and sample inlets. The microfluidic chips were mounted on a fluorescence microscope (Nikon Eclipse) to allow observation of cells passing through the device.

Supplemental **Figure 1.**



Supplemental Fig. 1 Experimental protocol for the determination of the effects of intracellular granules on leukocyte deformability profiles using paired donor samples.