High-Throughput Mechanobiology Screening Platform Using Micro- and Nanotopography

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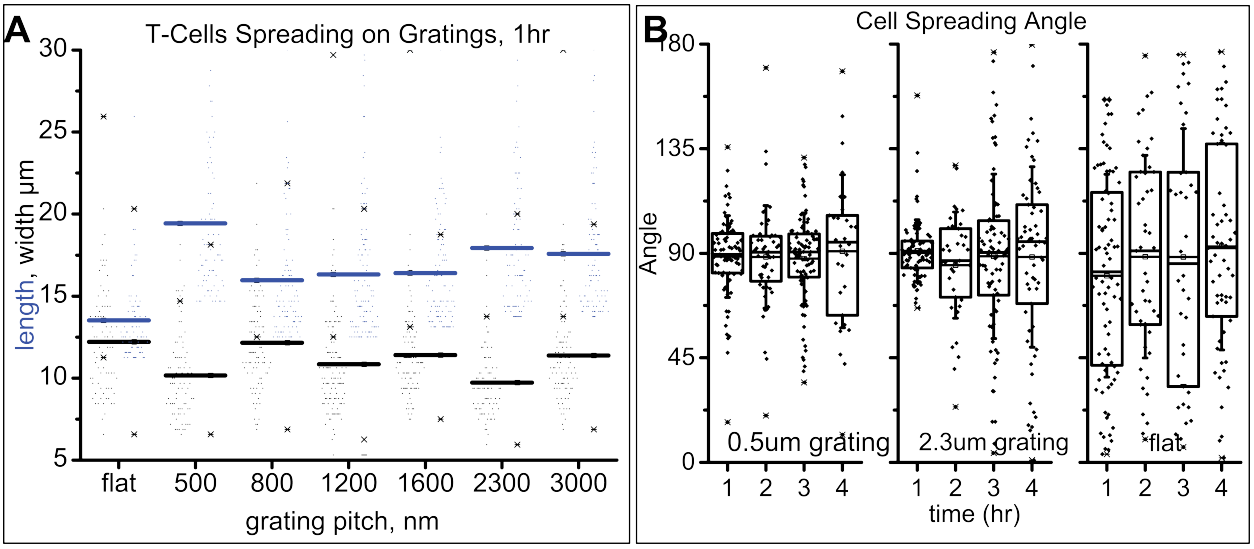
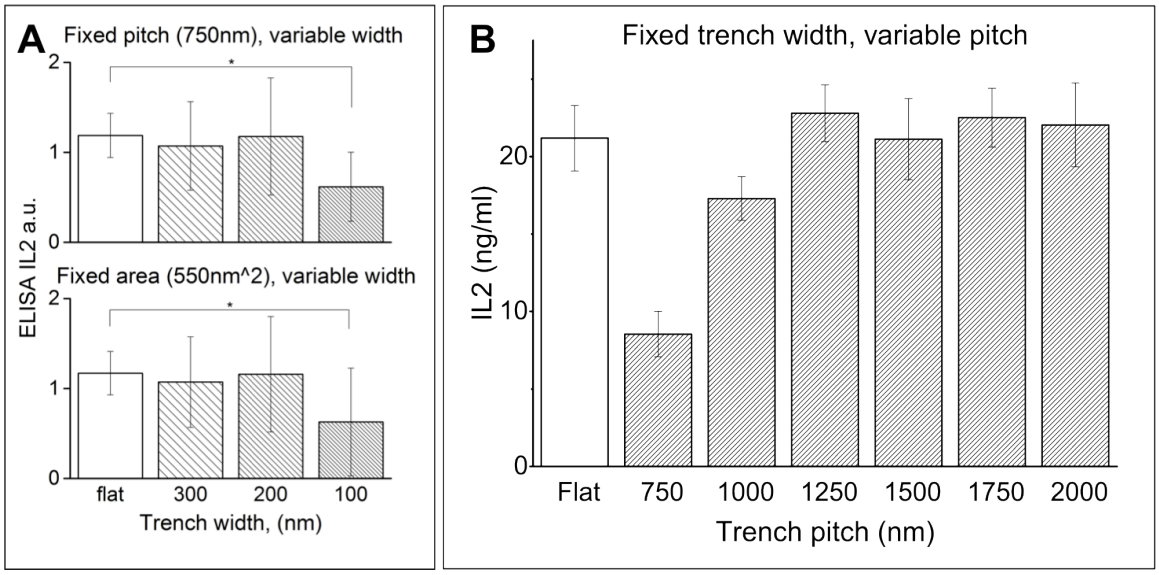
SUPPORTING INFORMATION

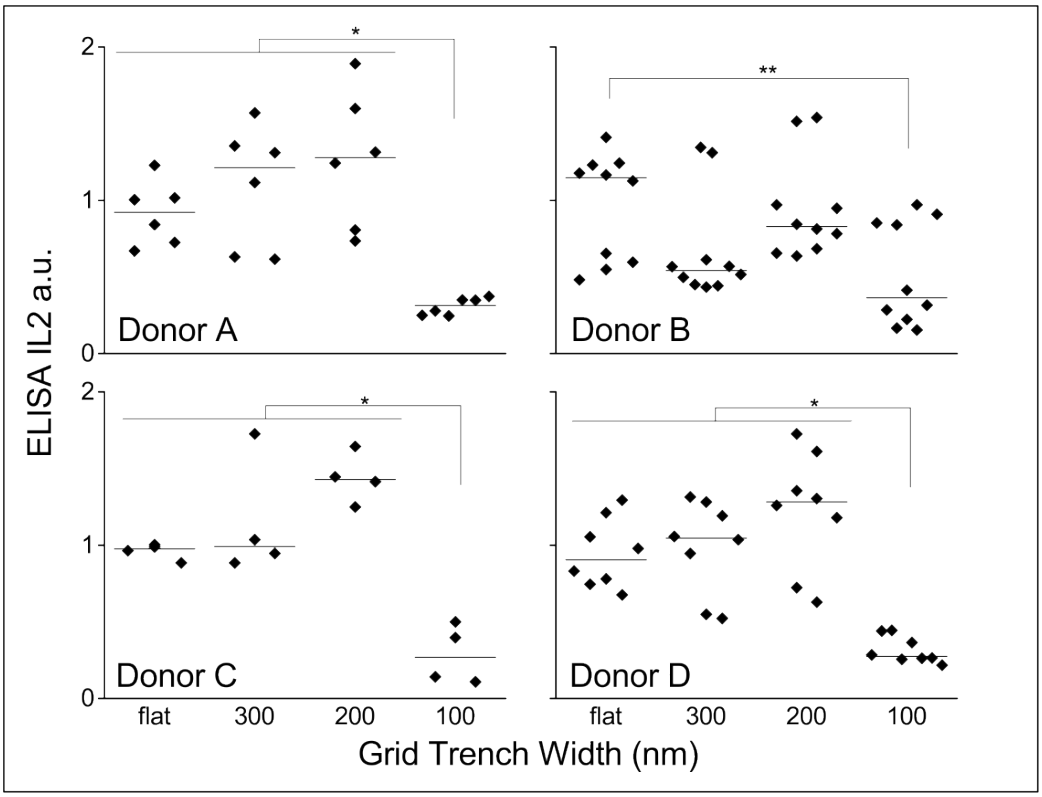
Supplementary Figures



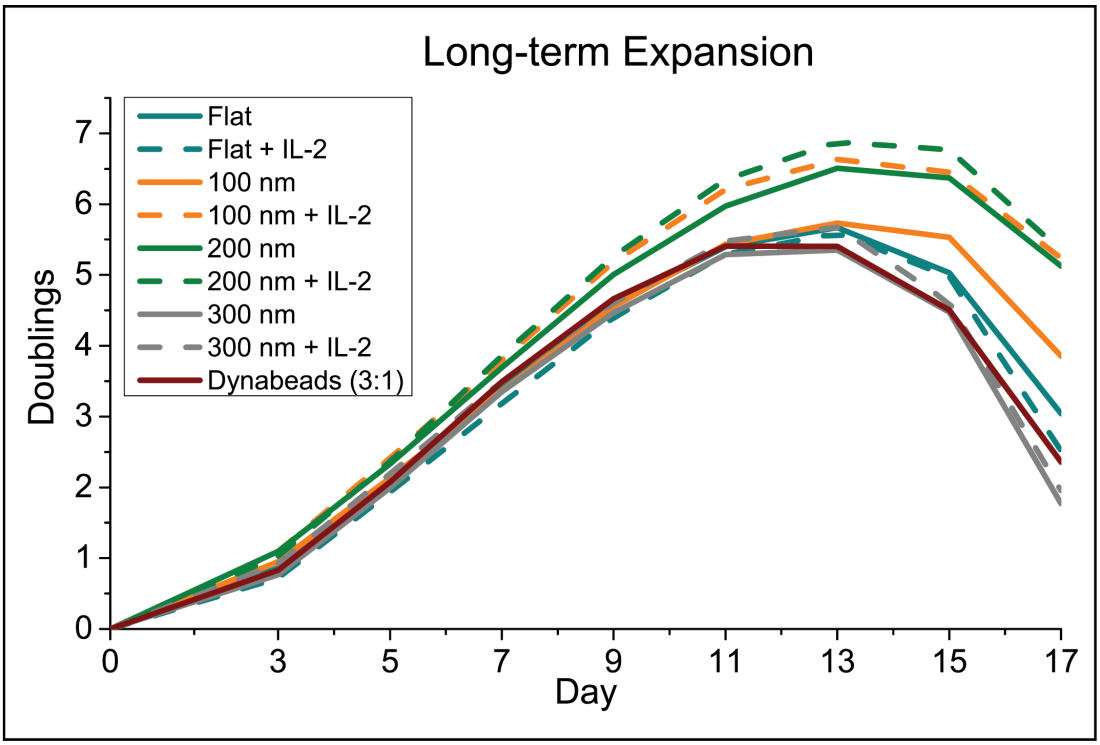
SFigure 1. A. T-Cells spreading on gratings are narrower, (width, black) and longer, (length, blue) compared to T-Cells on flat substrates, most pronounced on the smallest grating pitch (500 nm); B. The horizontal lines are population means. Cells initially are well aligned with the gratings, however after 4 hours of spreading the grating to cell alignment is observed to disintegrate.



SFigure 2. IL-2 secretion after 2 days, measured with ELISA, of T-Cells cultured on grid geometries with: A. fixed grid pitch and variable trench width and fixed area isolated between trenches and variable trench width; B. fixed trench width, variable trench pitch.



SFigure 3. Multiple donors(A-D) were tested on the grid geometry. IL-2 secretion enhancement on 200 nm trench width was observed for Donor A-C, the IL-2 secretion inhibition on 100 nm trench width was observed for all four different donors.



SFigure 4. T cell long-term expansion. T cell fold expansion is recalculated in a log scale. Highest doublings reaches to about 7 at day 13.

Methods

Pattern fabrication. SU8(2000-5) was spin coated on 4" silicon wafer at 3500rpm, 1500acel for 1min. The wafer was pre-exposure baked at 95°C on hot plate for 4mins. Designed patterns were written using electron bean lithography. After the Ebeam exposure, the wafer was put on hot plate for post-exposure baking at 75°C for 90 seconds (time critical). Then the resist is developed in SU8 developer solution for 1min. After washing with IPA, the wafer was put in oven at 100 °C to harden the SU8.

Pattern transfer. Patterned silicon wafer (500μm thick) with spin-coated hPDMS was put in the center of metal stage, with four 650μm thick shims at the four corners of the stage. Outgassed Sylgard 184 10:1 PDMS was poured in the center of the wafer. A polished Al plate was put on top and the drop of PDMS was pressed to the thickness of 150μm. Then the whole tool was put on hot plate at 100°C for 50mins. Patterned PDMS was peeled off after curing.

IMP assembly. 96 bottomless well plate was oxygen plasma treated for 1min and then soaked in 1.5% of APTES solution for 20mins. Then the plate was rinsed by DI water and N2 dried. Patterned PDMS substrate was plasma treated for 1min. Treated plate and PDMS were bonded under pressure for 30mins.

Cell thawing and seeding. Naïve CD4+ and CD8+ cell (isolated using RosetteSep isolation kits, Stem Cell Technologies) were previously frozen in -80°C freezer with 90% FBS and 10% DMSO solution. Cells were thawed in 37°C water bath and diluted with 37°C prewarmed 10ml X-VIVO full media (90% X-VIVO 15, Lonza; 5% Human Serum AB, Gemcell; 1% Glutamax, Invitrogen; 2% HEPES, Invitrogen; 1% Sodium Pyruvate, Invitrogen; 1% MEM Vitamin, Invitrogen) in 15ml centrifuge tube, tube was centrifuged at 4°C, 400rcf for 7mins. Supernatant was aspirated and the cell pellet was resuspended with 1ml full X-VIVO media. 0.5μl CFSE(CellTrace, Invitrogen) was added and stained for flow cytometry analysis. Another 9ml media was added before centrifuging process. After aspiration, the pellet was resuspended with 10ml media. Cells was split in 6 well plate and rested in incubator at 37°C in a humidified atmosphere with 5% CO2 for 12hrs before cell seeding. T-cells were seeded at the concentration of 0.8-1.0×10^6 cells/ml.

Antibodies coated substrate. 200μl oxidizing solution (DI:HCL:H_2O_2=5:1:1) was coated in the wells for 5 min. And the wells were washed with DI water for 3 times. Then washed by 95% of ethanol for 2 times. 200ul of 50% of APTES in ethanol solution was coated in the wells for 30mins. After aspiration, wells were washed by ethanol for 2 times and followed by DI water washing for 4 times. 0.5g dextran (Leuconostoc spp., Sigma) and 1g sodium periodate (Sigma) powder were dissolved in 20ml DI water, (stored at 4°C for overnight before use). 150μl of this dextran solution was coated in the wells for 1hr. Then wells were washed with PBS for 3 times. 200μl of 5% sodium periodate solution was coated for 2hrs. Then PBS washing for 3 times. 150μl Goat anti-Mouse(IGG,10ug/ml, MP Biomedicals) was coated for 30mins. Washed with

PBS for 3 times afterwards. 150µl OKT3 and 9.3 (anti-CD3, anti CD28, 2ug/ml, obtained from NYU, Dustin Group) was coated for 30mins. Washing with PBS for 3 times. Rested cell was taken from incubator and centrifuge down at 4°C, 400rcf for 7mins. Supernatant was aspirated and cell pellet was resuspended by 5ml fresh pre-warmed media. Cell count was obtained from hemocytometry. And cell concentration was diluted down to 0.8×10^6 cells/ml. 200µl cell solution was deposited in each culturing well.

Cell culturing and labeling. Cells were fixed with 4% Formaldehyde (F8775, Sigma) for 10mins and treated with 0.2% of Triton 100X (Sigma) for 4mins . Stained with Fluorescent Phalloidin (1:20, Invitrogen) for actin analysis and Hoechst 33342 (1:10,000, Invitrogen) for nucleus analysis. For flow cytometry, cells were labeled with Human CD4 APC Conjugate(1:10, Invitrogen), Human CD8 PerCp Conjugate(1:10, Invitrogen) and Propidium Iodine(~10ul per 50,000 cells, Invitrogen)

ELISA and flow cytometry. Supernatants were saved at variable time points from 12hrs to 3 days. Diluted by a factor of 25 to100. Plate coating used the standard protocol of Ready-Set-Go! ® ELISA (eBiosciense). IL-2 concentration was analyzed by Multiskan FC(Thermo Scientific) with two readings from 450 nm and 540 nm filters. Flow cytometery analysis was carried out on BD Biosciences FACSCanto II Flow Cytometer.

Long-term expansion. Following dextran coating, substrates were coated with goat-anti-mouse IgG (MP Biomedicals, 10 μg / mL, 0.5 h, room temperature) primary capture antibody, followed by a 1:1 mol:mol ratio of mouse-anti-human CD3 (BioXCell) / mouse-anti-human CD28 (BioXCell) (total 4 μg / mL, 0.5 h, room temperature). CD4+ / CD8+ cells were isolated from whole blood from healthy patients (Long Island Blood Center) and were seeded at 1 x 10^6 / mL

(200 k cells in 200 μL) (37 C, 5% CO_2). Cell proliferation was assessed beginning on day 3 post-seeding and every other subsequent day. Proliferation index was determined via carboxyfluorescein succinimidyl ester (CSFE, LifeTechnologies) nuclear staining on day 3 post-seeding with flow cytometry (BD FACSCantoII). Cells were frozen down for restimulation on completion of blasting phase, determined here as when the average cells volume dropped below 400 fL.

Restimulation. Following thawing, cells were allowed to rest for 12 hours. Cells were subsequently restimulated on Dynal beads (Human T cell activator, ThermoFisher Scientific) at a 3:1 ratio of beads:cells for 12 hours. IFNy staining (Miltenyi Biotec) was performed. Subsequent CD8 staining (Biolegend) was performed by incubating cells with 5 μg / mL of stain for 20 minutes at 4 ºC. Cells were fixed (2% PFA, 10 min, 4 ºC), and readouts were performed via flow cytometry (BD FACSCantoII)