Lab on a chip

Supplementary information

- **Title:** Emulating endothelial dysfunction by implementing early atherosclerotic microenvironment within a microfluidic chip
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Figure S1 Schematic of experimental setup for the flow and imaging system.



Figure S2 (A) Cellular orientation angle relative to the fluid flow axis after the cells were exposed to shear stress for 25 hours. (*** $p \le 0.0001$) (Box and whisker plot: range of the box = 25 ~ 75%, line in the box = median value, red square in the box = mean value, and whisker = \pm 0.5 IQR) (B) Aspect ratio of cells within the monolayer after the cells were exposed to shear stress for 25 hours. (*** $p \le 0.0001$) (Box and whisker plot: range of the box = 25 ~ 75%, line in the box = median value, red square in the box = mean value, and whisker = \pm 0.5 IQR) hours. (*** $p \le 0.0001$, ** $p \le 0.001$) (Box and whisker plot: range of the box = 25 ~ 75%, line in the box = median value, red square in the box = mean value, and whiskers = \pm 1.5 IQR). Number of cells for all experimental conditions are ~20,000.



Figure S3 Measuring VE-cadherin width in collagen, 19.2 kPa VE-cadherin image: (A) A representative image showing how to select a perpendicular line across the VE-cadherin signal. (yellow line), (B) black line: the intensity profile across the yellow line in A, blue line: Gaussian fitting. Measuring VE-cadherin width in fibronectin 19.2 kPa VE-cadherin image: (C) A representative image showing how to select a perpendicular line across the VE-cadherin signal. (yellow line), (D) black line: the intensity profile across the yellow line in C, blue line: Gaussian fitting. Scale bar = 25 μ m

After fitting the VE-cadherin signal intensity to the Gaussian curve, we can measure the full width and half maximum (FWHM) using the following formulas. We considered FWHM as the width of the VE-cadherin.

Gaussian function:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} exp\left[-\frac{(x-x_0)^2}{2\sigma^2}\right]$$

Full width and half maximum:

FWHM $\approx 2.355 \sigma$



a. Dextran layer (above cells or gel surface)

b. Polyacrylamide gel layer (below cells or gel surface)

Figure S4 Representative side view images of PA gel (19.2 kPa) with and without cellular monolayer immersed in FITC-dextran. These side view images are reconstructed using 3D project plugin (using the projection method "mean value") in ImageJ software. Every z-section is 5 um apart and between z-sections the signals are interpolated.