Electronic supplementary information (ESI)

Real-time quantification of endothelial response to shear stress and vascular modulator

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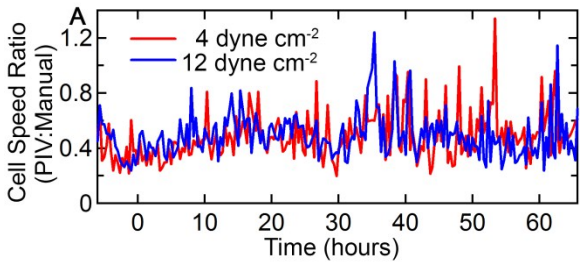
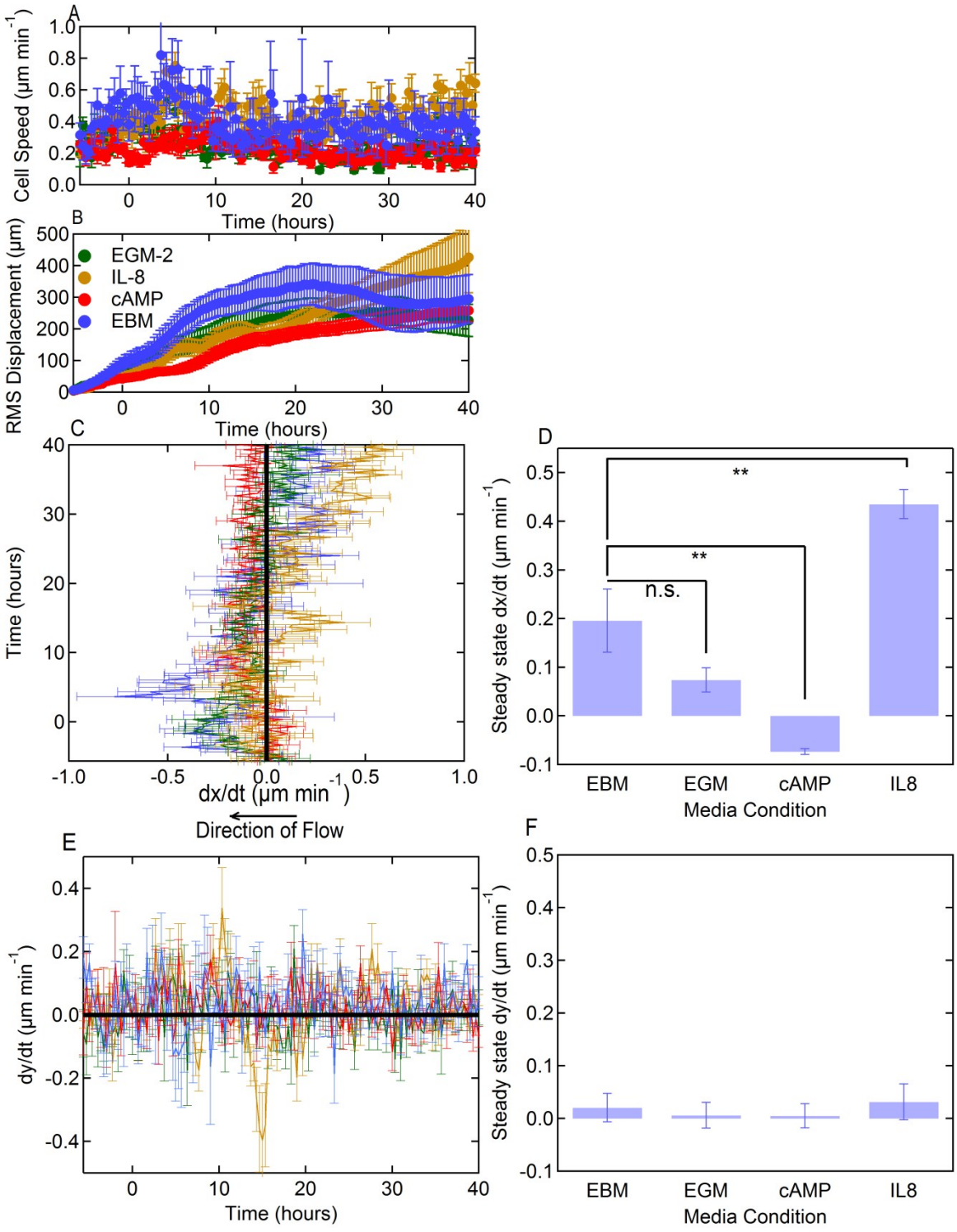
Comparison of cell speed from PIV and manual cell tracking



Supplemental Figure 1. Cell speed ratio (PIV:manual) as a function of time calculated using OpenPIV software (PIV) and manually tracking cell position (manual). PIV cell speed is calculated using OpenPIV software to automatically approximate the cell speed within the endothelial monolayer. To verify the accuracy of the cell speed obtained using the PIV algorithm, individual cells within the monolayer were manually tracked. Briefly, the location of the nucleus was identified and used to calculate cell speed based on the nuclear shift between each frame and plotted as a function of time ($n > 10$). Comparison shows that the PIV output ($\mu m\ min^{-1}$) is typically a factor of 2 smaller than the cell speed obtained through manual cell tracking.



Supplemental Figure 2. RMS displacement and directional bias for confluent monolayers under 16 dyne cm^{-2} shear stress in various media conditions. (A) Average cell speed as a function of time. (B) Average RMS net displacement as a function of time and referenced to the initial position at the beginning of flow. (C) Average X speed as a function of time. (D) Steady state X directional bias. (E) Average Y speed as a function of time. (F) Steady state Y

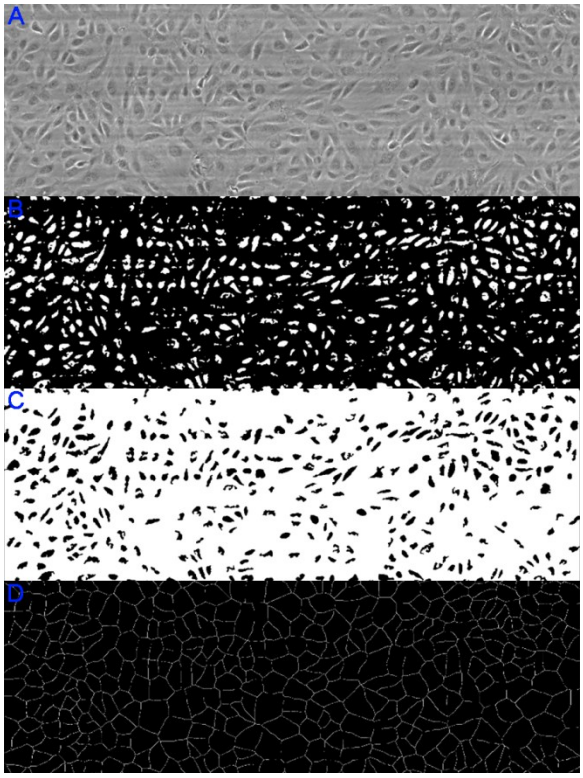
directional bias. The separate X and Y components for each cell's translational speed was averaged respectively during the steady state regime (30 - 40h). Directional bias is the average cell speed assessed separately in the X or Y direction, over a given length of time (i.e. 30 - 40 h). Flow is the in the negative X direction, right to left. All data were obtained from manually tracing at least n = 7 individual cells for each of the media conditions. Values represent mean ± SE. * $p < 0.05$, ** $p < 0.01$. Not significant (n.s.).

The RMS displacement shows that cells move relatively large distances, typically 200 – 400 μm during a 40-hour experiment; however, there is no statistical difference between any of the media conditions. To assess any directional bias, we measured the X- and Y-components of the displacement in each of the 20 minute intervals. The results show that cells in IL-8 at steady state migrate significantly faster than those in EBM against the direction of flow ($p = 0.006$), whereas those in EGM-2 are not significantly different from EBM ($p = 0.108$). Cells in cAMP at steady state migrate with the direction of flow (**Supplemental Table 1**).

	EBM	**EGM-2**	**IL-8**	**cAMP**
Avg. X Speed (μm min^{-1})	0.19	0.07	0.44	-0.07
Stand. Dev	0.19	0.07	0.08	0.02
n	9	7	7	7
P-value	0.014, *	0.021, *	1.7 x 10^{-6}, ***	3.9 x 10^{-6}, ***
Avg. Y Speed (μm min^{-1})	0.02	0.005	0.03	0.006
Stand. Dev	0.08	0.06	0.09	0.06
n	9	7	7	7
P-value	0.465	0.837	0.387	0.812

Supplemental Table 1. Steady state directional bias. Position and speed were manually tracked every 20 minutes for n number of cells as shown in **Supplemental Figure 2**. The separate X and Y components for each cell's translational speed was averaged respectively during the steady state regime (30 - 40h). For each condition, the time average X and Y directional speeds across n cells were compared to zero speed (i.e. random walk) using a one-sample t-test; p-values are reported for a two-tailed t test. Positive X-speed is against the direction of the flow while negative is with the direction of the flow. We observe significant directional bias (i.e. not equal to zero) for each media condition along the X direction, parallel to flow, and no significant bias in the Y direction, perpendicular to flow.

Cell tracing



Supplemental Figure 3. Representative images of cell tracing. (A) Phase contrast image of a confluent endothelial cell monolayer. (B) Thresholded image of monolayer highlighting the cell nuclei. (C) Inverted the images (D) Final cell trace.

Images of cell monolayers from time-lapse movies were imported into ImageJ and the cell borders were delineated automatically using a custom macro.[17] At each time point, images from the three locations within respective channels were analyzed and averaged. Experiments with different media conditions were performed in triplicate. Morphological parameters (inverse aspect ratio, area, and orientation angle) of individual cells were obtained as long as more than 85% of the monolayer could be traced by this method.