Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplemental information

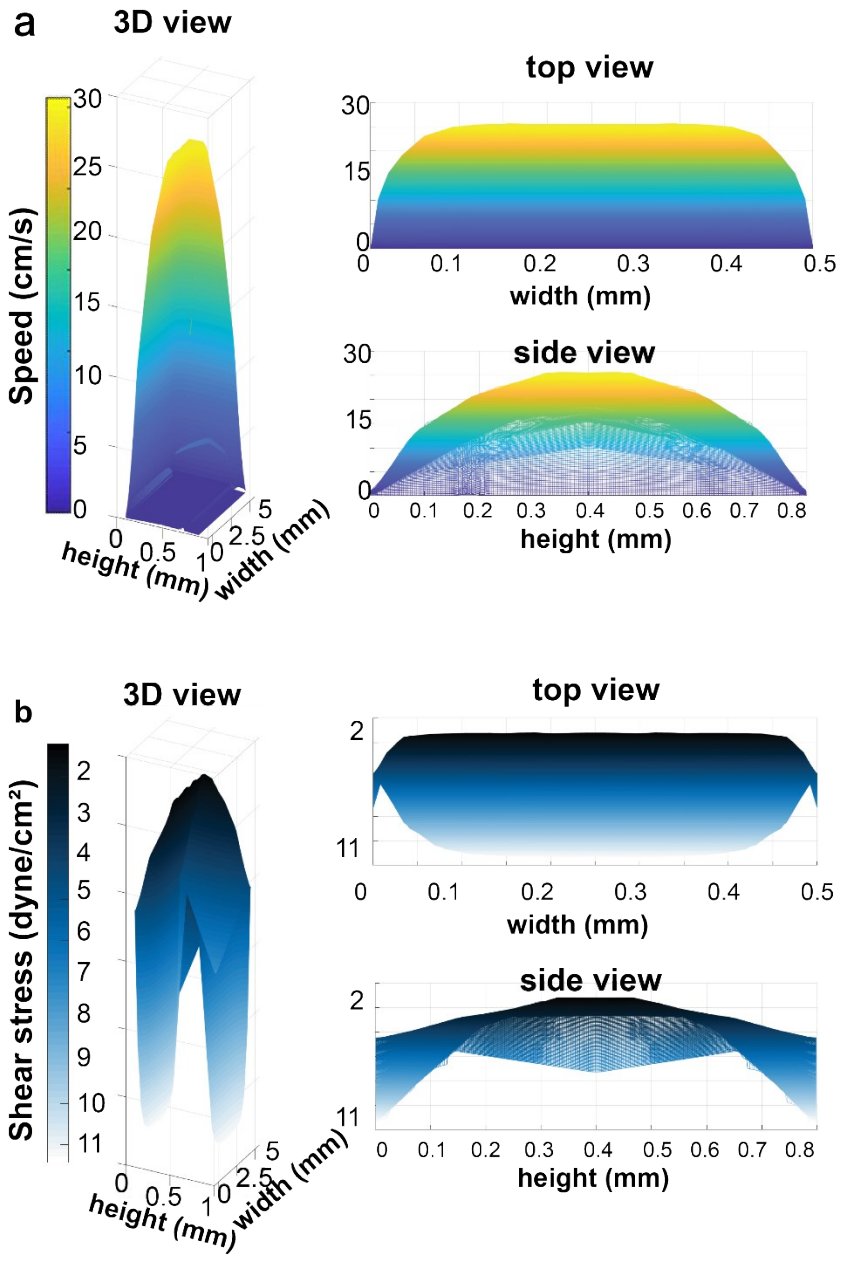

Figure S1. Shear stress and speed simulations. 3D plot of shear stress and speed simulations at 800 mbar pressure using the dimensions of a 0.8 mm ibidi µ-slide.

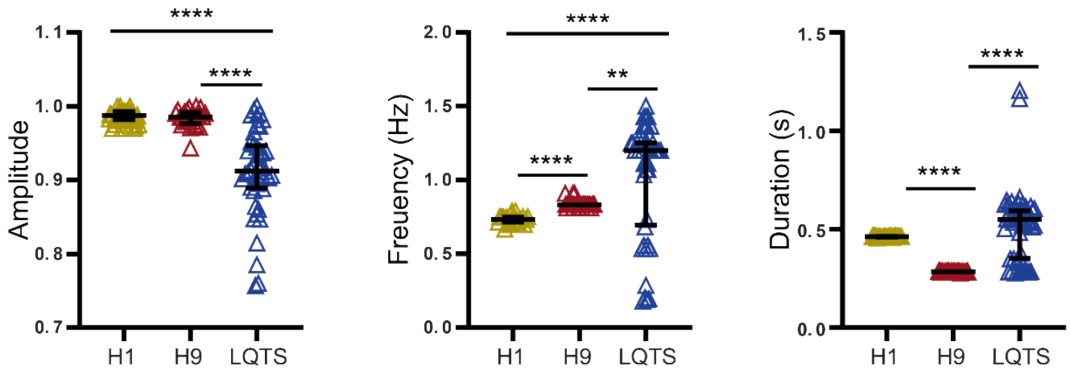

Figure S2. Characterization of different hPSC-CS contraction waveforms. Normalized contraction amplitude, contraction frequency, and contraction duration are presented as mean ± SEM. Each hPSC-CS contraction waveform was calculated from the individual contractions over 60 seconds. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests. $^{**}p < 0.01$, $^{****}p < 0.0001$.

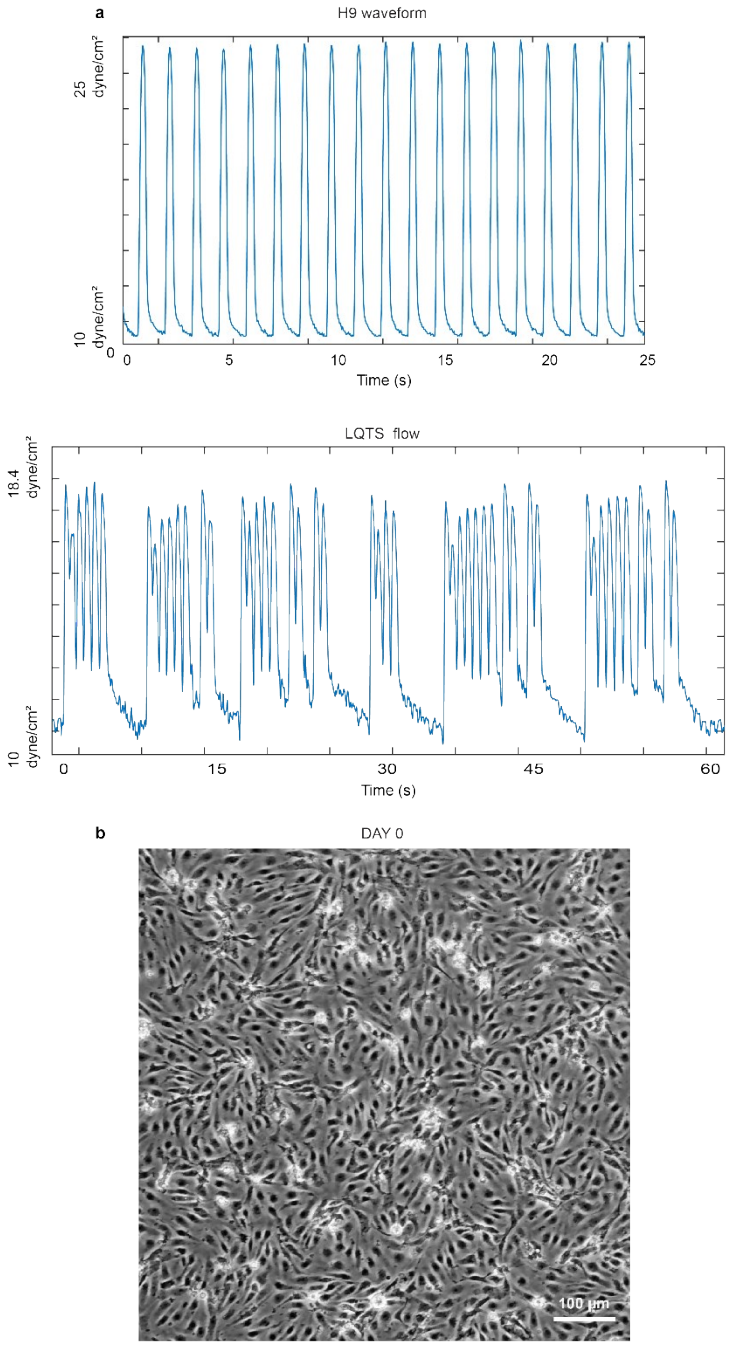

Figure S3. Pulsatile flow profile applied to EC differentiation and brightfield images of EC after differentiation under shear stress. (a) Pulsatile flows applied to EC progenitors during differentiation. H9-CS derived pulsatile flow and LQTS-CS derived pulsatile flow applied to the EC progenitors were maintained at the baseline of 10 dyne/cm^2. The peak shear stress for H9-CS

derived pulsatile flow was 25 dyne/cm^2 and the peak shear stress for LQTS-CS derived pulsatile flow was18.4 dyne/cm^2. The average shear stress levels for both hPSC-CS derived pulsatile flows were at 13.7 dyne/cm^2. **(b)** Representative brightfield images of EC progenitors (DAY 0) Scale bar, 100 μm.

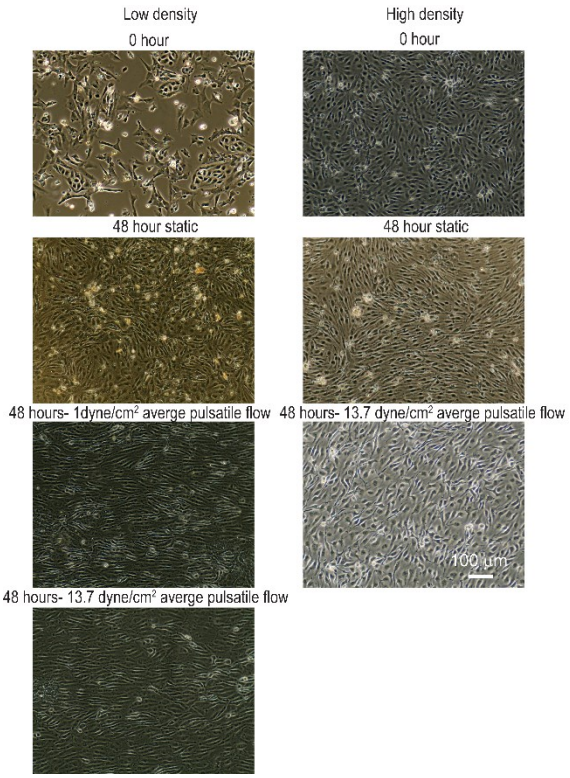

Figure S4. Brightfield images of ECs after differentiation under static and shear stress conditions. Pulsatile flows or static conditions were applied to EC progenitors at low density (left, non-confluent cells) and high density (right, confluent cells). After 48 hours, images under static condition, 1 dyne/cm^2 average pulsatile flow, and 13.7 dyne/cm^2 average pulsatile flow were taken by brightfield microscopy. Scale bar, 100 μm.

Pressure (mbar)	Measured shear stress (dyne/cm^2)	Measured shear stress +Max uncertainty (dyne/cm^2)	Percent error
200	0.923	1.000	8.82
150	0.693	0.764	10.27
100	0.436	0.455	4.42
50	0.108	0.111	2.48

Supplemental Table 1. Uncertainty analysis of shear stress.

The uncertainty analysis of shear stress considered velocity error due to 1) observed bead size, 2) measured streak length, 3) bead diameter error, and 4) streak length error due to imaging depth of field, at all z-depths (800, 700, 600, 500 μm) for all 4 measured pressures (50, 100, 150, 200 mbar) included in the study. Shear stress was calculated 5 μm below the top of the ibidi μslide at the channel-liquid interface at (z = 795 μm).

Group	**Pearson's r value**
H1 ES-CS	0.96
H9 ES-CS	0.96
LQTS iPSC-CS	0.98
LQTS-CS before nifedipine	0.79
LQTS-CS after nifedipine	0.83
H9-CS before verapamil	0.97
H9-CS after verapamil	0.95

Supplemental Table 2. Pulsatile waveforms and their corresponding contraction waveforms are highly correlated. Pearson correlation was performed to verify the cross-

correlation between pulsatile waveforms and their corresponding contraction waveforms. R values indicated.

Supplemental videos: 21 supplemental videos