Live imaging of nitric oxide release in vascular endothelial cells in response to mechanical stimuli on an organ chip

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Background: Nitric oxide (NO), released from vascular endothelial cells in response to mechanical stimuli, regulates cardiac contractility and are also involved in the prevention of the development of cardiac hypertrophy.
Purpose: To establish an experimental system for live observation of NO release in response to mechanical stimuli on an organ chip.
Methods: Organ chips, which we used for the development of a heart-on-a-chip in the previous study [1], were used.
We seeded 300,000 human umbilical vein endothelial cells on a stretchable elastic membrane coated with Matrigel of a chip channel. Shear stress was applied to the cells by increasing flow rate of a peristaltic pump connected to the chip channel (Figure 1A). Pressure stimulus was applied by hydrostatic pressure. Stretch stimulus was applied by suction to the side ports of a chip using an electric syringe pump (Figure 1B). Cells were stained with 10 μM 4,5-diaminofluorescein diacetate for fluorescent live NO imaging.
Results: Monolayers of the endothelial cells formed intercellular junctions confirmed by CD31 staining (Figure 1C, yellow). Apparent permeability, which was measured by Texas red dye (MW 3000), was maintained at a low level of $\sim 3 \times 10^{-6}$ cm/s until day 30, suggested the formation of robust intercellular junction.
When the endothelial cells were subjected to a pressure stimulus of 60 mmHg for 60 s, NO release was observed that lasted for >2 minutes (Figure 2A). A peak value of 1.46±1.08 (mean ± standard deviation) times the baseline was observed 271 s after the beginning of the pressure stimulus (n=251 cells). When the cells were subjected to a 1% stretch for 60 s, a peak value of 1.29±0.33 times the baseline was observed 105 s after the beginning of the stretch stimulus (Figure 2B). A shear stress of 0.01 dyn/cm^2 hardly increased NO release (1.20±0.27 times the baseline, Figure 2C).
Conclusion: The system for live NO imaging in vascular endothelial cells in response to mechanical stimuli was established using organ-on-a-chip. The heart-on-a-chip with endothelial cells will be useful in elucidating the effects of mechanical stimulus such as hypertension on the contractile function and the remodeling of the heart.

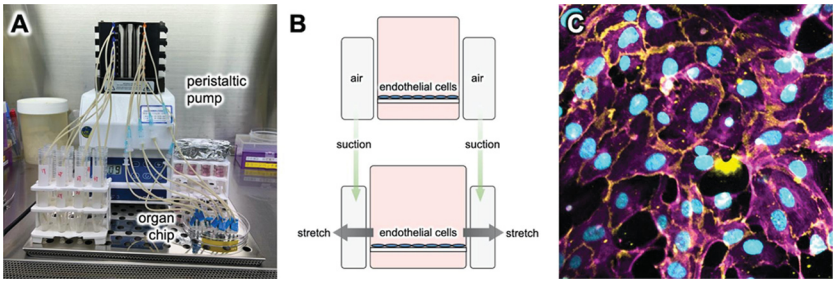

Figure 1

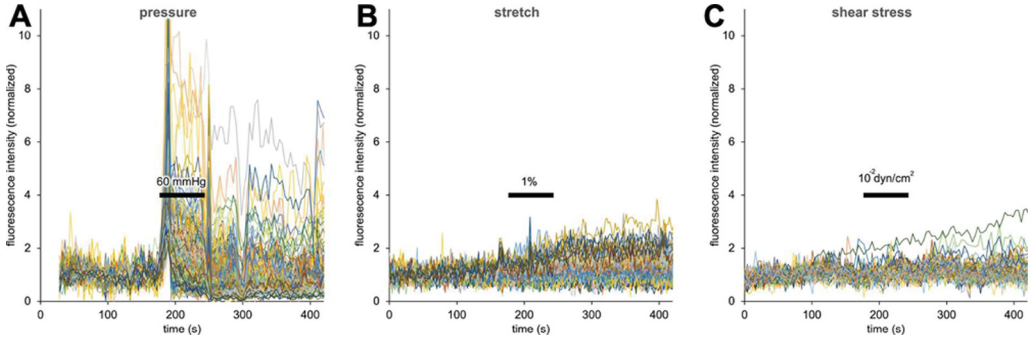

Figure 2