Supporting Information

Multi-corneal barriers-on-a-chip to recapitulate eye blinking shear stress forces

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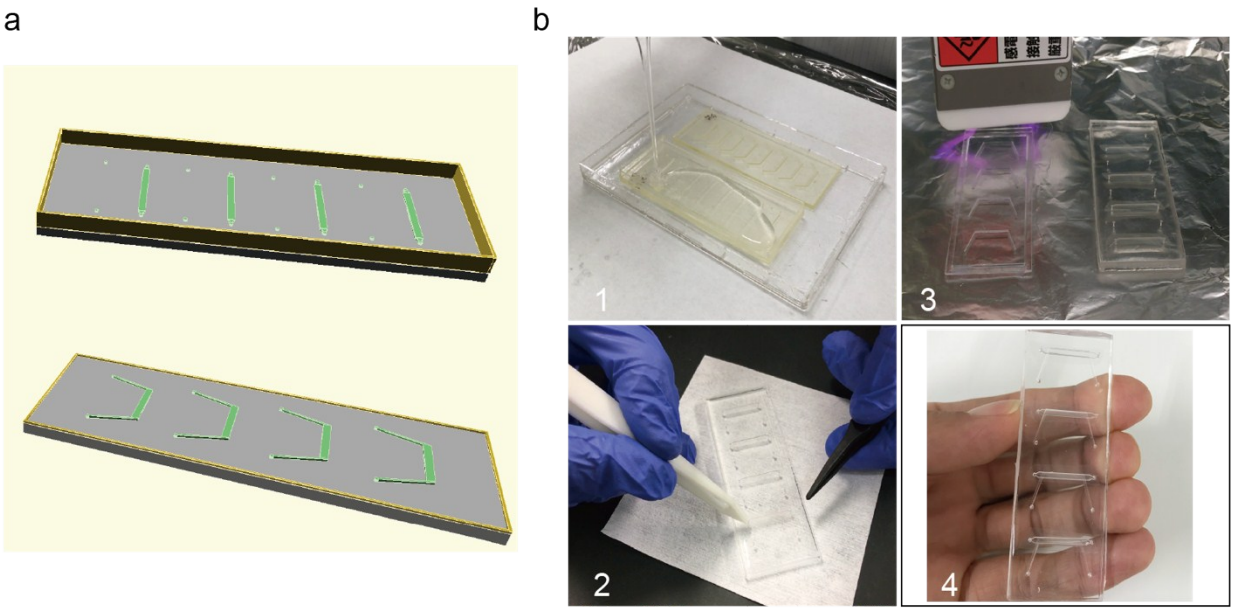
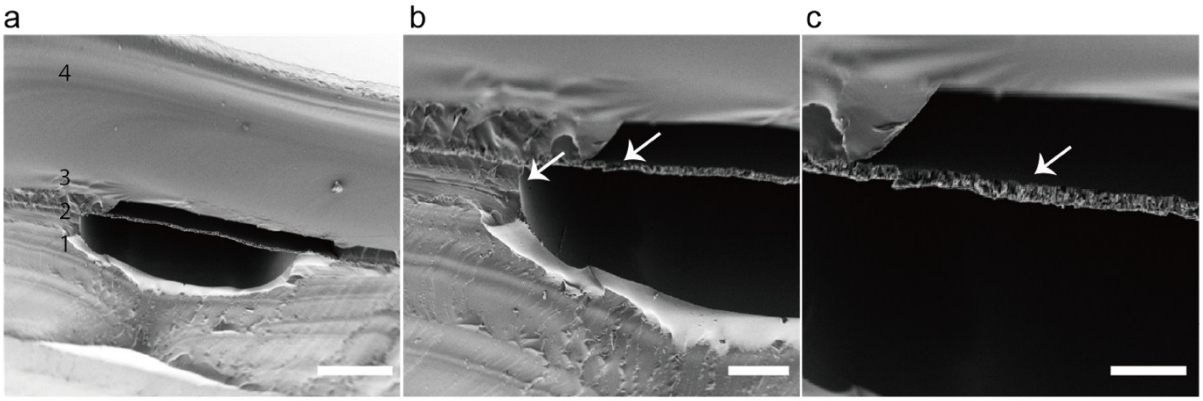
Supplementary Table. 1 The aspect ratios of HCE-T cells under static and flow dynamic conditions. Data represent as mean ± SD of three independent experiments. *P*-Value was determined by the Nested T test.

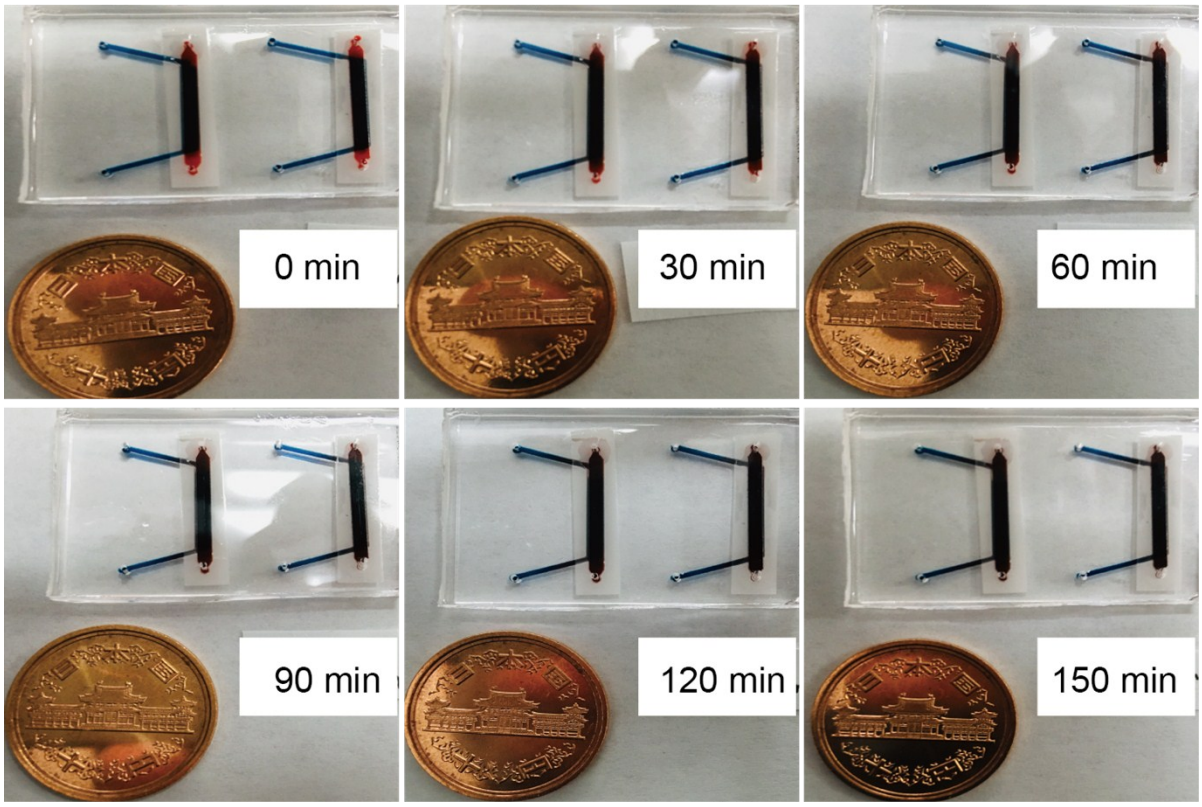
	STATIC			DYNAMIC			P-VALUE
AREA	A1	A2	A3	A1	A2	A3	<0.001
ASPECT RATIO	1.476±0.32	1.467±0.32	1.458±0.30	1.503±0.35	1.508±0.36	1.514±0.35	



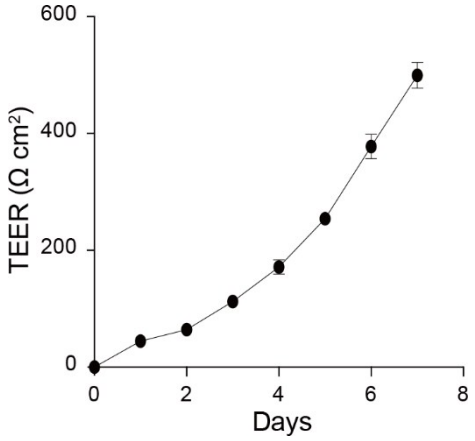
Supplementary Fig. 1 The design and fabrication of the multi-well microfluidic device. a, Design of the lower and upper channels with OpenSCAD. **b,** The process of fabrication; [1)] The addition of PDMS and curing at 80 °C for overnight [2)] The insertion of PET membranes in between the upper channels and lower channels [3)] Bonding with corona treatment [4)] The final structure of the multi-well microfluidic device**.**



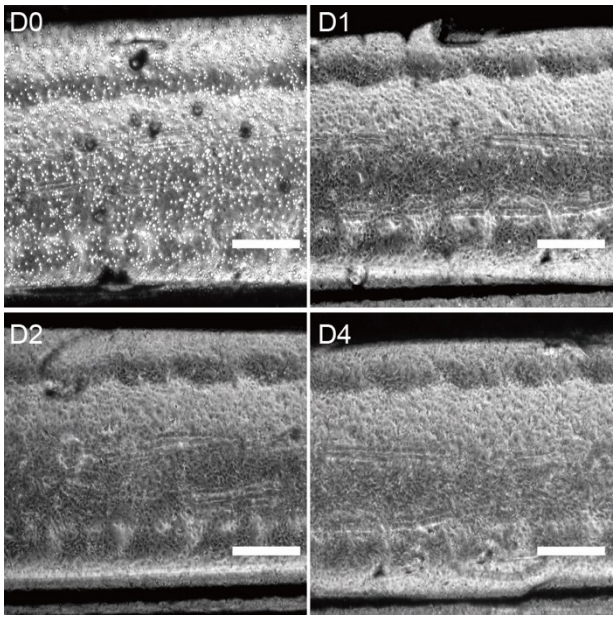
Supplementary Fig. 2 SEM images of a cross-section of the microfluidic channel. a, The structure of the microfluidic channel [1)] Upper PDMS channel [2)] PET porous membrane [3)] Lower PDMS channel [4)] Glass slide. Scale bar, 500 µm **b and c,** The intactness of the porous membrane after fabrication. Arrows indicates no gaps between upper and lower channels. Scale bar, 200 µm; 100 µm respectively.



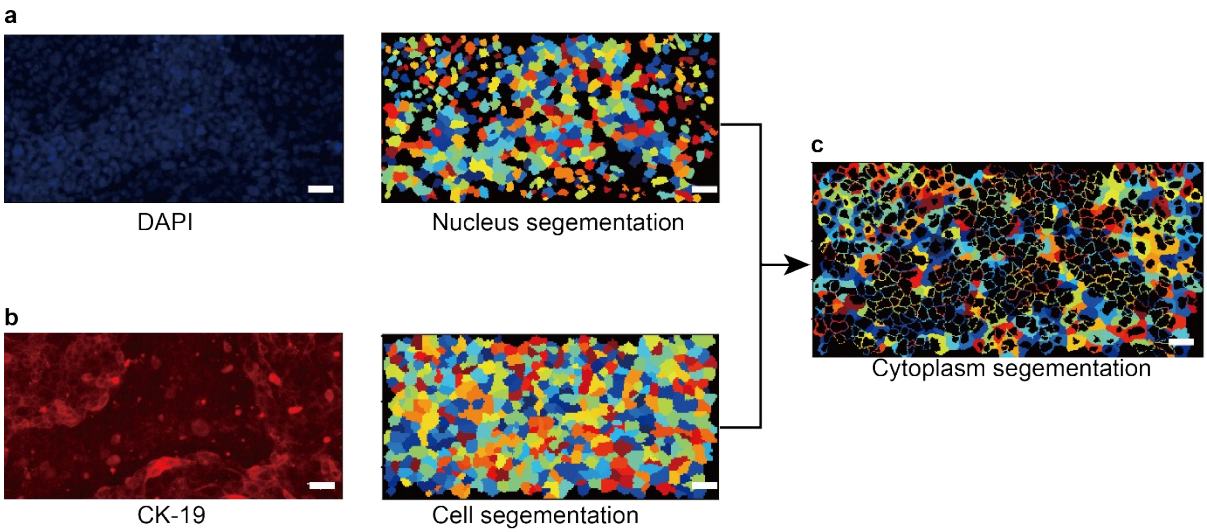
Supplementary Fig. 3 Dye leakage and translocation in the multi-well microfluidic device.



Supplementary Fig. 4. TEER measurement of the corneal epithelial barrier in trans-well system. HCE-T cells were seeded in trans-well in a density of 0.89×10^5 cells cm^{-2} for 7 days. Data represent as mean ± SD of eight independent experiments.



Supplementary Fig. 5. Brightfield images of HCE-T cells post-seeding the multi-well microfluidic devices. HCE-T cells were seeded in the microchannels in a density of 6×10^3 cells cm^{-2}. Scale bar, 500 µm.



Supplementary Fig. 6. Single cell analysis by using CellProfiler. The process consisted of four steps: a, The identification of the primary nucleus objects by using the Otsu's segmentation of the primary nucleus based on their diameter and the fluorescence intensity of the nucleus stain DAPI. b, The identification of the secondary cells body objects by using CK-19 fluorescence localization. c, The identification of tertiary cytoplasm objects through the measurement of the difference among nucleuses and cells. And finally, the morphological features of cells descriptors were exported for further analysis. Scale bar, 50 μm.