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

Electronic Supplementary Information

Dielectric Elastomer Actuator for Mechanical Loading of 2D Cell Cultures

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Strain uniformity in the electrode gap

Using the device surface topography and digital image correlation (DIC), we measured the device displacement field upon actuation, and calculated the corresponding strain profile. The measurement was made at 4 kV, which corresponds to an average strain of ε_{yy}=11%, thus reproducing the strain profile experienced by LECs in the reported experiment. The *y*-strain ε_{yy} is presented in Fig. S1(a), the *x*-strain ε_{xx} is presented in Fig. S1(b), and cross-sections of the strain profiles are presented in Fig. S1(c)-(d). The results show good strain uniformity on the electrodes, and most of the gap.

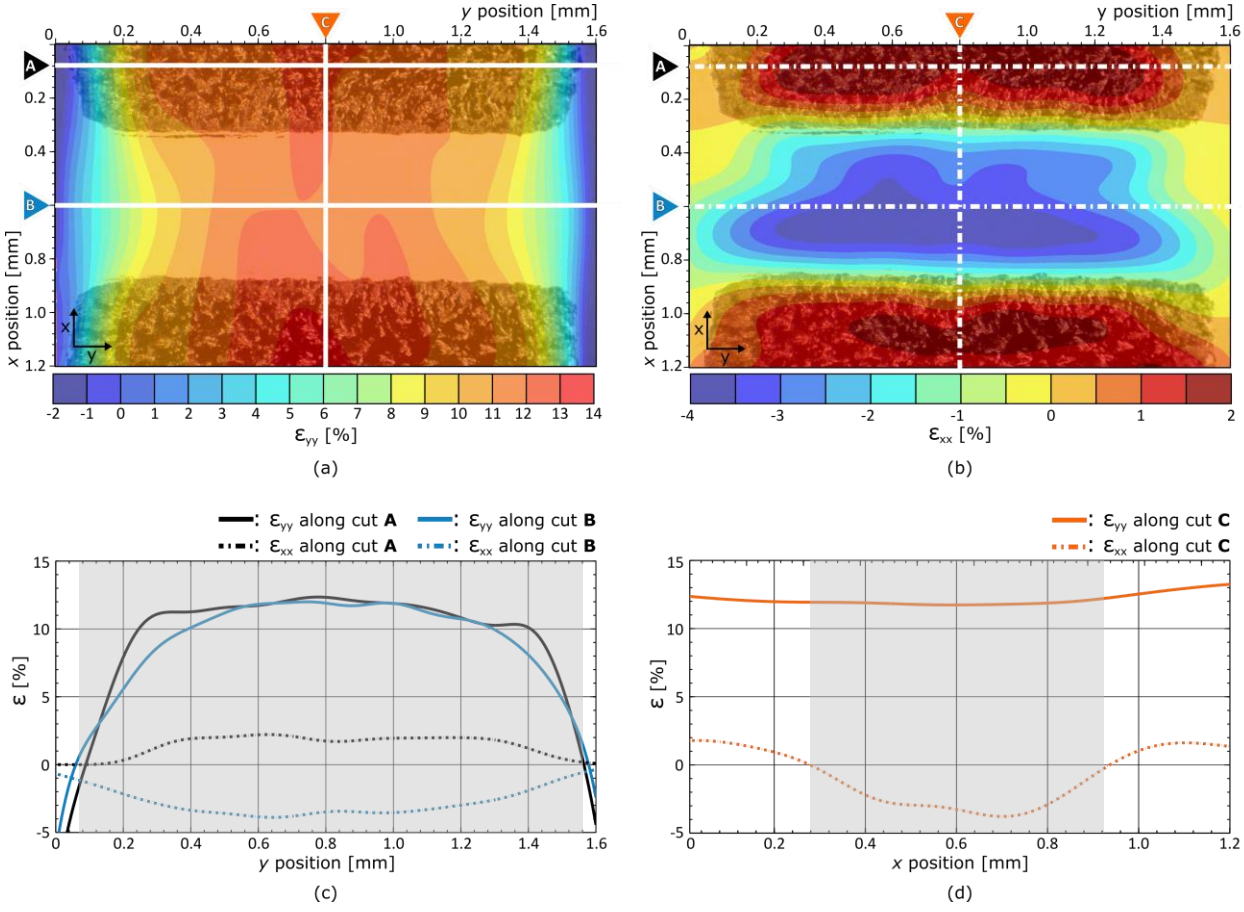

Figure S1: Using the device surface topography and digital image correlation (DIC), we measured the displacement field in the electrode gap, and calculated the corresponding strain profile. (a) The *y*-strain ε_{yy} profile is overlaid on a picture of the device. (b) The *x*-strain ε_{xx} profile is overlaid a picture of the device. (c) ε_{yy} and ε_{xx} profiles along the cuts **A** and **B**, with the gap region highlighted in grey. (d) ε_{yy} and ε_{xx} profiles along the cut **C**, with the gap region highlighted in grey.

Effect of fringing electric field on LECs

This section details the control experiment we designed in order to assess the effect of the device fringing electric field on lymphatic endothelial cells (LECs) morphology. The electric field generated by the actuator is mostly confined within the membrane, but cells located at the border of the electrode are however not perfectly shielded. In order to confirm that morphological changes we observed on LECs upon stretching are not induced by fringing electric field, we repeated the stretching experiment with an immobilized device. In order to suppress the actuation we replaced the oil backing by a glass slide directly bonded to the membrane. Figure S2 presents the actuation strain of the DEA-based deformable bioreactor before and after immobilization. The cells on the immobilized device are exposed to the same electric field, while not being mechanically stimulated, effectively decoupling the electric field exposure from the mechanical stimulation. The experiment described in the paper was repeated with an immobilized DEA-based deformable bioreactor. The device was connected to a high-voltage power supply and cycled between 0 V and 4.3 KV at a 0.1 Hz frequency with a 50% duty cycle for 24 h. The actuation voltage of 4.3 kV corresponds to an electric field of 145 V/µm, and generated 10% strain before immobilization, replicating the experimental parameters of the reported experiment.

Figure S3(a)-(b) presents fluorescence micrographs acquired in the stimulated and control areas of the device after the 24 h experiment. Signals obtained from the DNA and VE-cadherin (AF1002, R&D Systems) stainings are shown in blue and red, respectively. The DNA is concentrated at the nucleus and can be used to identify and count cells, whereas VE-cadherin is concentrated at the cells junctions and can be used to characterize cell morphology. The results show no visible difference in cell morphology between the stimulated and control cells.

Figure S3(c) presents the orientation distribution with respect to the strain axis. Green ellipses and black arrows on the left schematize cells and the strain axis respectively. The results show a random orientation of LECs in the stimulated and control areas. Figure S3(d) presents the elongation distribution. The elongation coefficient corresponds to the ratio between the long and short axis of a cell, and the green ellipses on the left schematize the corresponding shapes. The results show similar distribution in the stimulated and control areas.

This control experiment demonstrates that fringing electric field of the DEA-based deformable bioreactor has no effect on LECs morphology, and indeed confirms that the reported LECs orientation and elongation was induced by the DEA periodic mechanical stimulation.

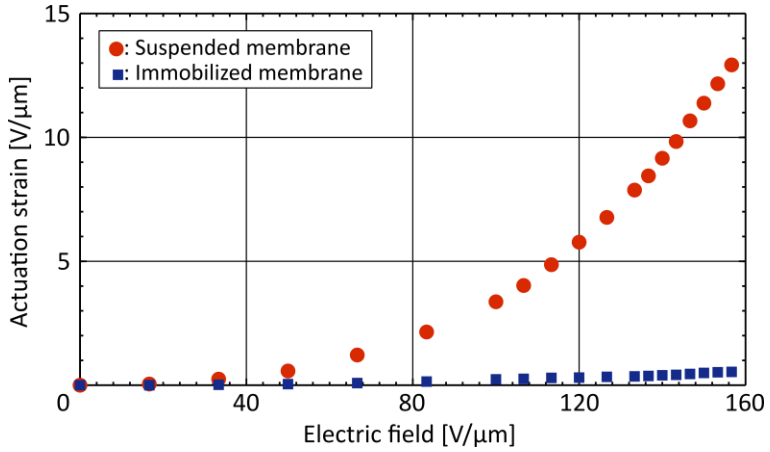

Figure S2: Actuation strain of our DEA-based deformable bioreactor before and after immobilization. The actuation strain at 145 V/µm decreases from 10% for the suspended membrane, to 0.5% for the immobilized membrane. The immobilization was achieved by replacing the oil backing by a glass slide directly bonded to the membrane.

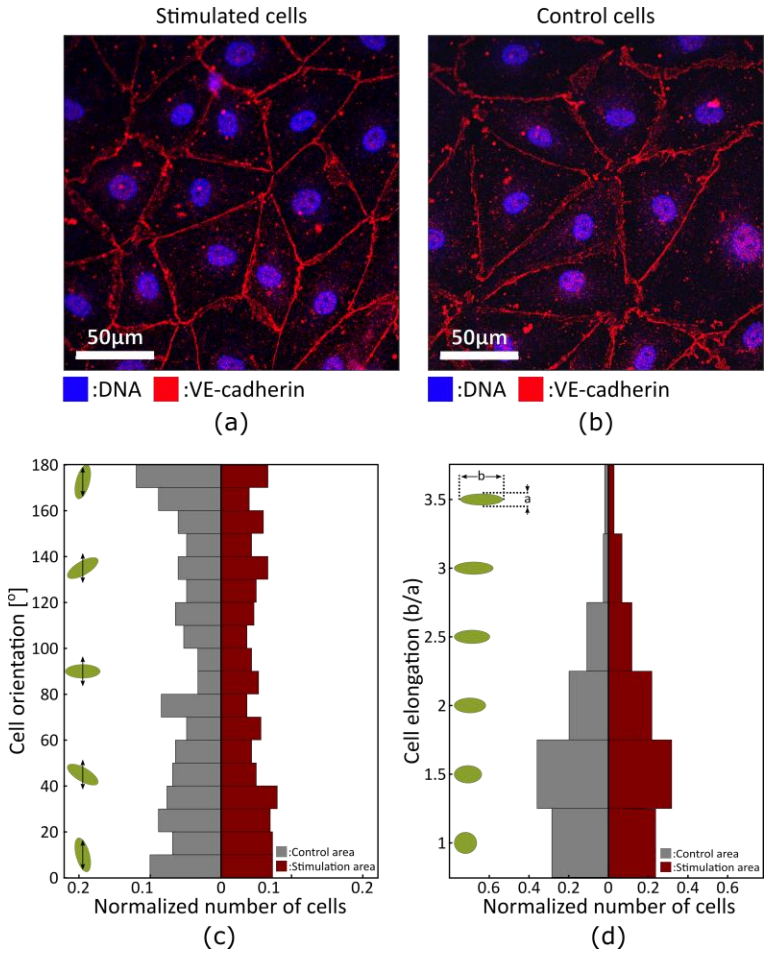

Figure S3: Fluorescent micrographs acquired in the (a) stimulated and (b) control areas show no difference in cell morphology. Measurements were done after 24 h of cyclic actuation between 0 V/µm and 145 V/ µm (corresponds to 10% strain before immobilization) at a 0.1 Hz frequency. (c) Orientation distribution of cells in the control and stimulated areas. The green ellipses and the black arrows schematize the cells and the strain axis respectively. Results show random orientation in both areas, confirming that the device fringing electric field has no effect on LECs morphology. (d) Elongation distribution of cells in the control and stimulated area. Elongation is calculated as the ratio between the long and short axis of cells, and the green ellipses schematize the corresponding cell shapes. Results show similar distribution in both areas, confirming that the device fringing electric field has no effect on LECs morphology.

Dynamic optical monitoring

Our DEA-based deformable bioreactor enables real-time optical monitoring of the cell monolayer. The in-plane actuation mode of DEAs provides very good out-of-plane stability during actuation, and the oil backing minimizes sagging of the membrane under the weight of the growth medium. The time-lapse video shows a LECs population being stretched by 10% at a 0.1 Hz frequency under a 10X microscope objective lens (EC Plan-Neofluar 10x/0,30 Ph1 M27 (WD=5,5mm)). The out-of-plane stability of the systems allows for the cell monolayer to passively remain in focus during stretching.