Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Supplementary Information

Small-Artery-Mimicking Multi-Layered 3D Co-Culture in Self-Folding Porous Graphene-Based Film

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Supplementary captions for

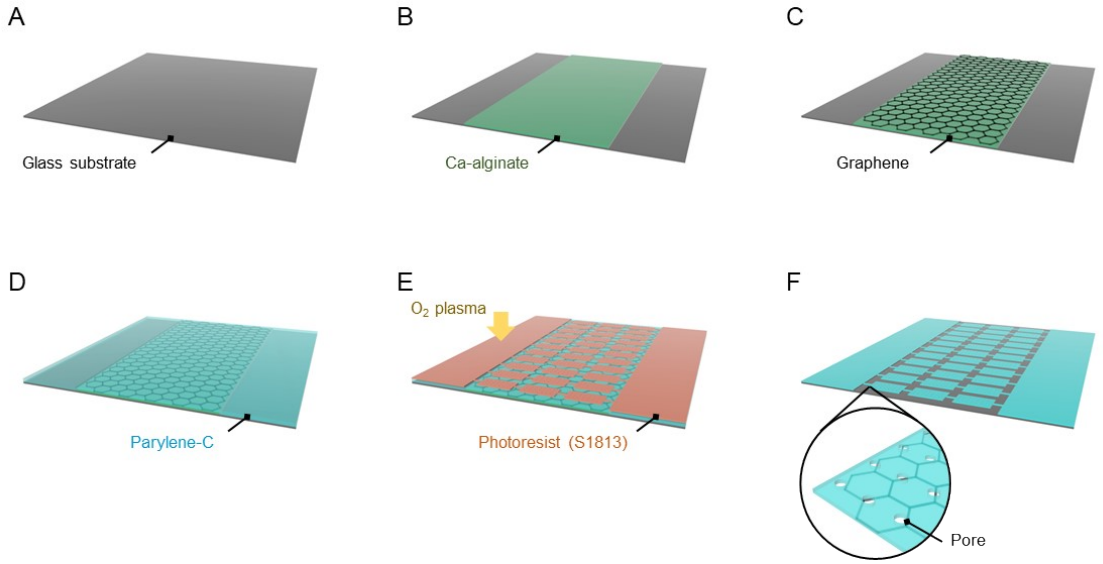

Fig. S1 Fabrication process of self-folding film. (A) (B) A 6×18 mm Ca-alginate layer was formed on an 18×18 mm glass substrate. (C) A graphene monolayer was transferred onto the Ca-alginate layer. (D) Parylene-C was deposited on the substrate to form the parylene-C layer using the CVD process. (E) The layered film was etched by RIE with O_2 plasma through the photoresist mask. (F) The array of rectangle self-folding films was formed. The rectangle self-folding films were mechanically supported by the cross-shaped hinges. Note that both sides of the film don't detach from the substrate even after dissolving the sacrificial Ca-alginate.

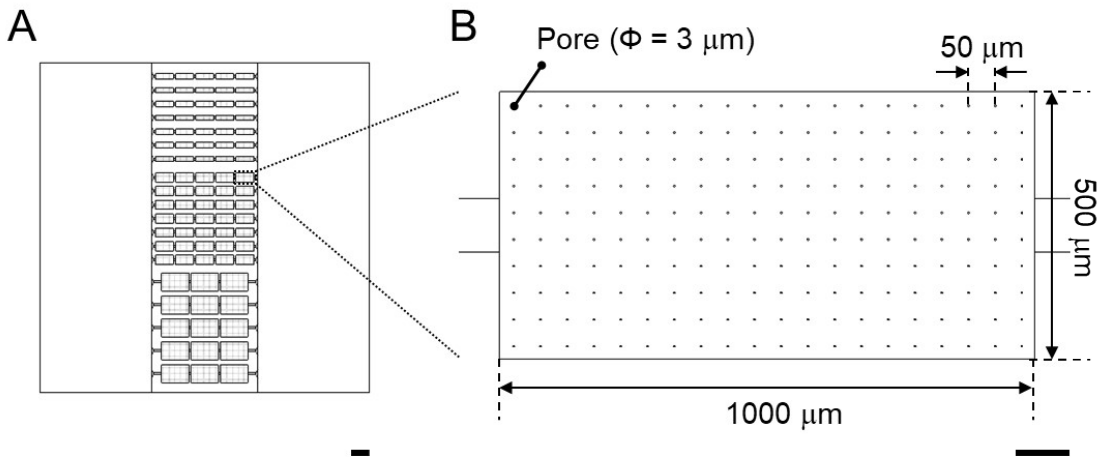

Fig. S2 Micro-pattern of self-folding film. (A) A representative pattern of self-folding film array. (B) The rectangle self-folding film. The width of film was varied from 300 to 1000 μm. The length of film was varied from 1000 to 1500 μm. Scale bar indicate 1 mm (A) and 100 μm (B).

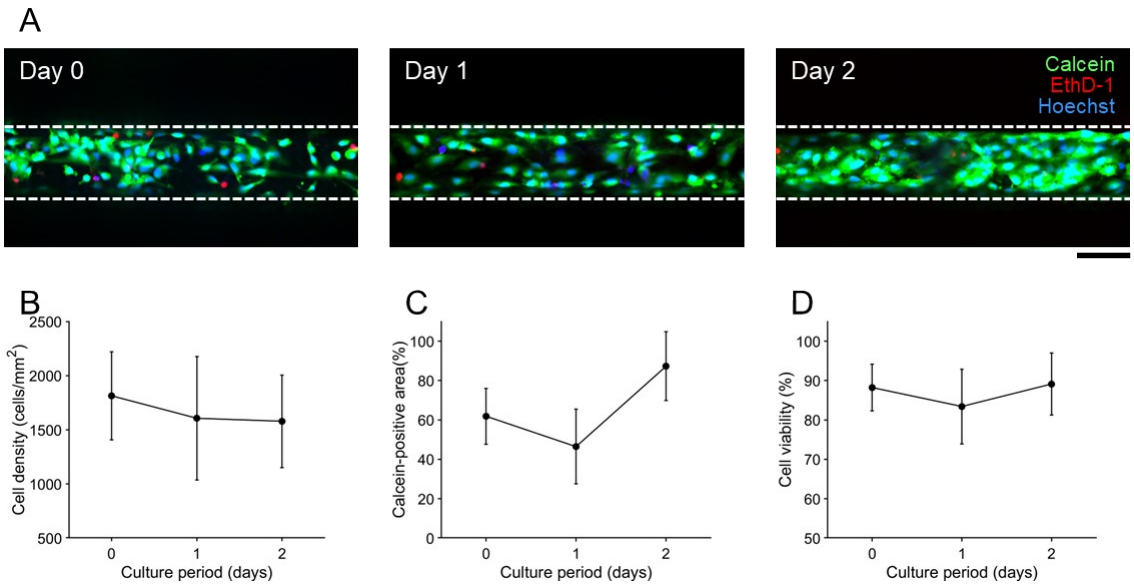

Fig. S3 Live/dead staining of HUVECs cultured on micro-rolls. (A) live/dead staining images of HUVECs using calcein AM (live cell; green), Ethidium homodimer-1 (EthD-1; dead cell; red), and Hoechst 33342 (nuclei; red). White dotted lines indicate the edge of the micro-roll. Scale bar indicates 100 μm. (B-D) The graph shows cell density (B), calcein-positive area (C), and cell viability (D) at different days. The cell density is calculated from the number of live cell nuclei. The calcein-positive area indicates the ratio of binalized calcein-positive pixels to the region of interest, which is indicated by two white dotted lines. The cell viability is the ratio determined by dividing the number of live cell nuclei by the total number of cells. Note that the cell density decreased one day after seeding due to cell migration from inside to outside of the micro-roll, while HUVECs extended their cell bodies and formed a confluent monolayer on the inner surface of the micro-roll after two days of culturing. Additionally, the viability assay shows that there was no significant cell death inside the micro-roll. $n >$ 20 micro-rolls.

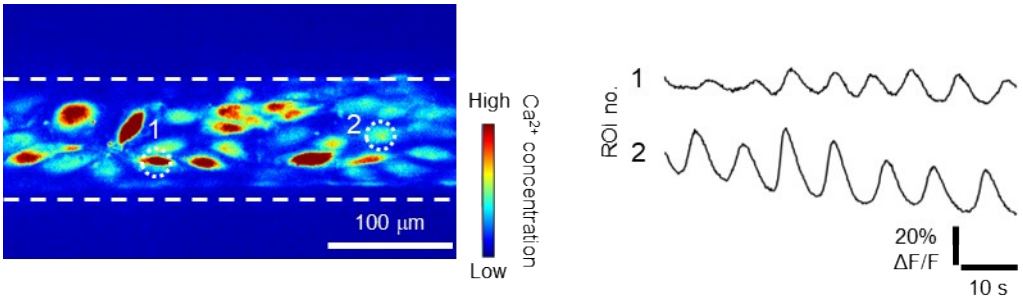

Fig. S4 Calcium transients in encapsulated HUVECs. The intracellular Ca^{2+} concentration of HUVECs encapsulated within the micro-roll was observed using Fluo-8, calcium-sensitive dye. The intracellular Ca^{2+} concentration in HUVECs that were cultured in the culture medium containing VEGF spontaneously oscillated. The Ca^{2+} oscillations in different HUVECs were not synchronized, suggesting that the Ca^{2+} transient was occurred by external substances.

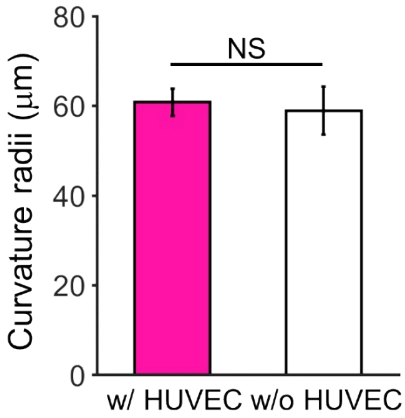

Fig. S5 The effect of cell-culture on the curvature of micro-roll. Curvature radii of micro-rolls with and without culturing HUVECs were obtained. There is no significant differences in the curvature between two conditions. n > 25 micro-rolls. NS: not significant.

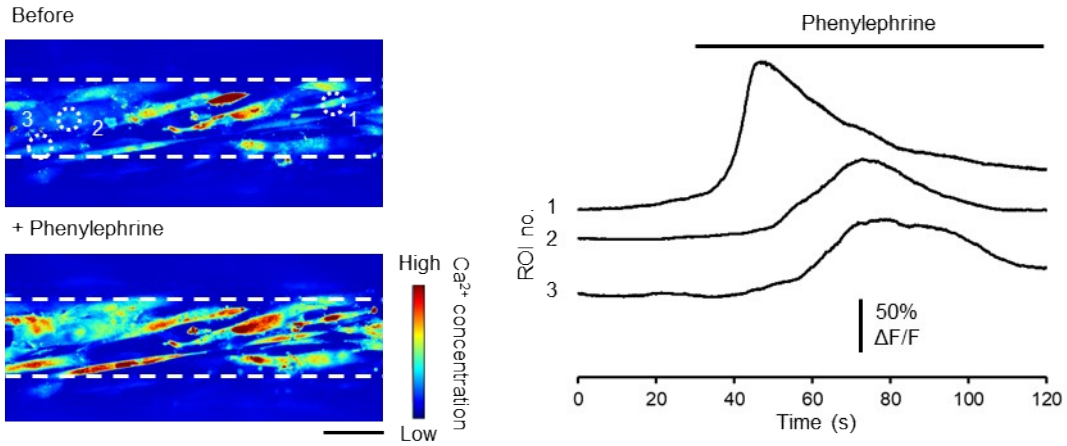

Fig. S6 Calcium responses in HUASMCs to application of phenylephrine. The intracellular Ca^{2+} concentration in HUASMCs was monitored using Fluo-8 before and after the adding 10 μM phenylephrine to the medium. The intensity profiles were obtained from the region of interests (ROI) indicated by the white dotted circles in the heatmap image. The intracellular Ca^{2+} concentration was dramatically increased by the application of phenylephrine. Scale bar indicates 100 μm.

Supplementary Movies

Movie S1 Time-lapse images of HUVECs inside the micro-roll. Time-lapse phase-contrast images of HUVECs encapsulated within the micro-roll were acquired using an all-in-one fluorescence microscope (BZX700; Keyence) at 2 frames per an hour for 48 h.

Movie S2 Time-lapse images of HUVECs and HUASMCs co-culture. Time-lapse phase-contrast images of co-cultured HUVECs and HUASMCs in the micro-roll were acquired at 2 frames per an hour for 48 h.