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

Electronic Supplementary Information

Transient microfluidic compartmentalization using actionable microfilaments for biochemical assays, cell culture and organs-on-chip

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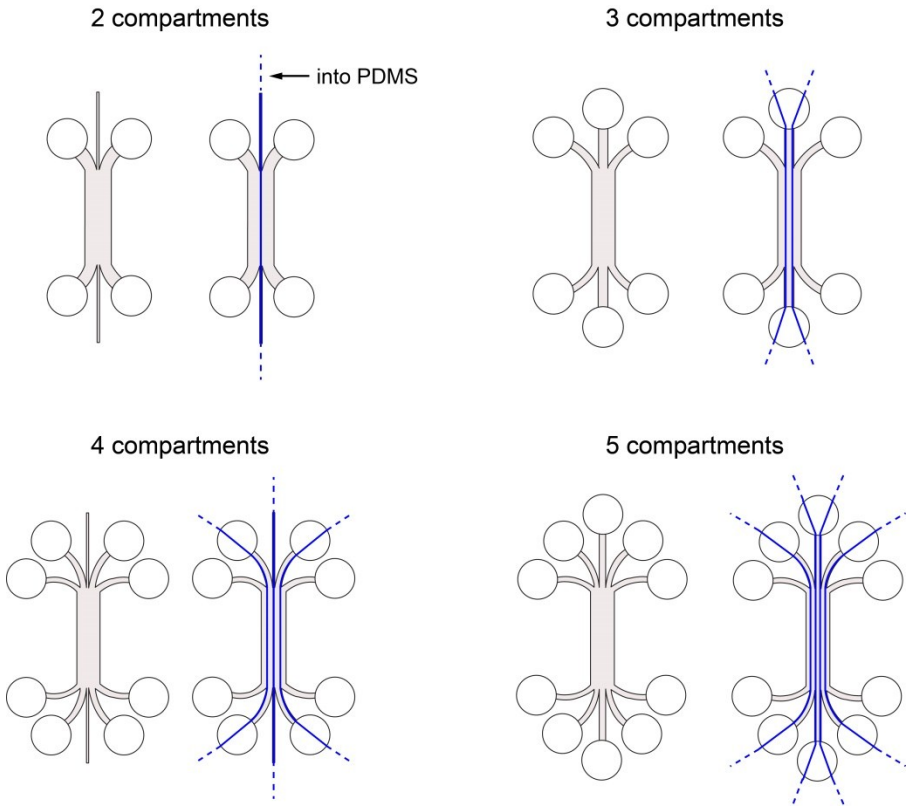


Figure S1. Designs of microfluidic chambers for integrating 1 to 4 partitions.

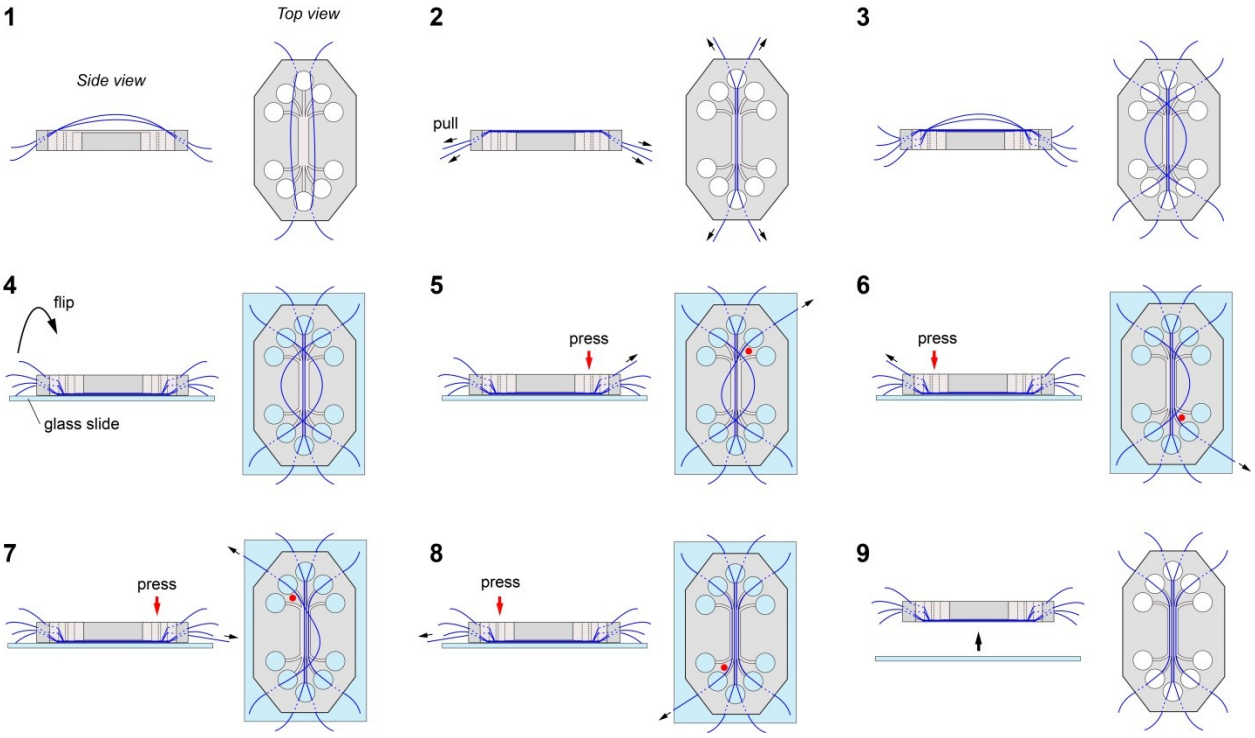


Figure S2. Scheme of the insertion of 4 microfilaments. Middle 2 filaments were partially embedded in the PDMS part using a sawing needle and tensed along the middle channel (1, 2). Two other filaments were introduced, sandwiched between the PDMS part and a glass slide, and tensed along curved channels step by step while points close to the channel, indicated as a red point in the top view figures, were pressed down (3-8). After all the filaments were well tensed, the PDMS part was detached and used for the experiments (9).

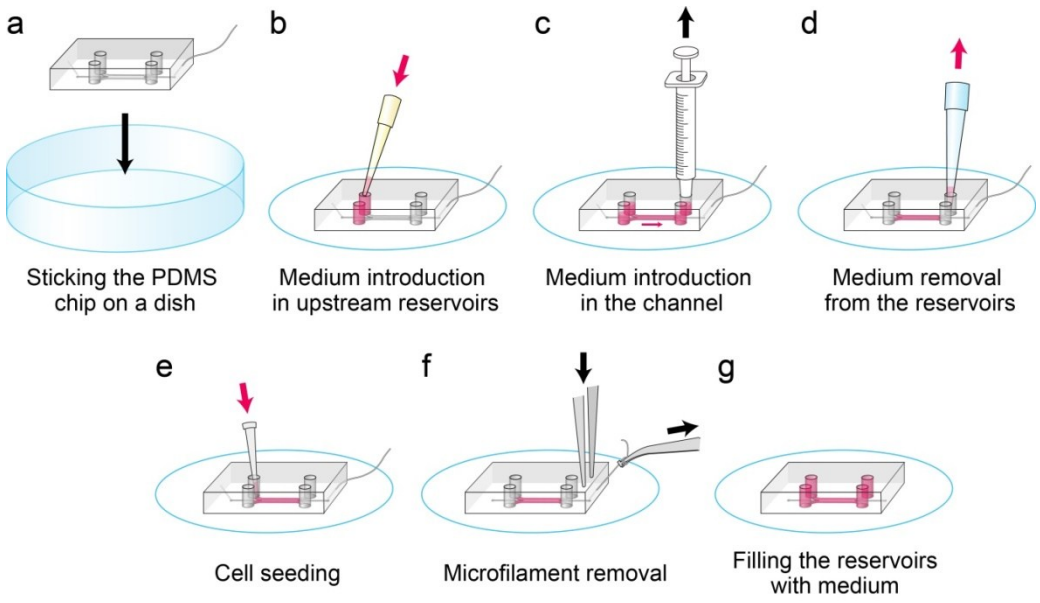


Figure S3. Chip assembly with a cell culture dish as a bottom substrate without plasma bonding. (a) The PDMS part of the device, without plasma treatment, was pressed down on the bottom of a cell culture dish. (b) Two upstream reservoirs were filled from the bottom with 40 µL each of culture medium. (c) The medium was introduced in the chamber by sucking from downstream reservoirs using a 1 mL disposable syringe. (d) Medium was removed from all the reservoirs by micropipette prior to cell seeding. (e) 3 µL each of cell suspension was introduced in the chamber from the upstream reservoirs by micropipette. (f) One hour after cell seeding, the microfilament was removed using 2 pairs of tweezers: the device was pressed down with 1 pair of tweezers, while the filament was removed by using 1 pair of bent tweezers. (g) All the reservoirs were filled with 40 µL each of culture medium for further cell culture.

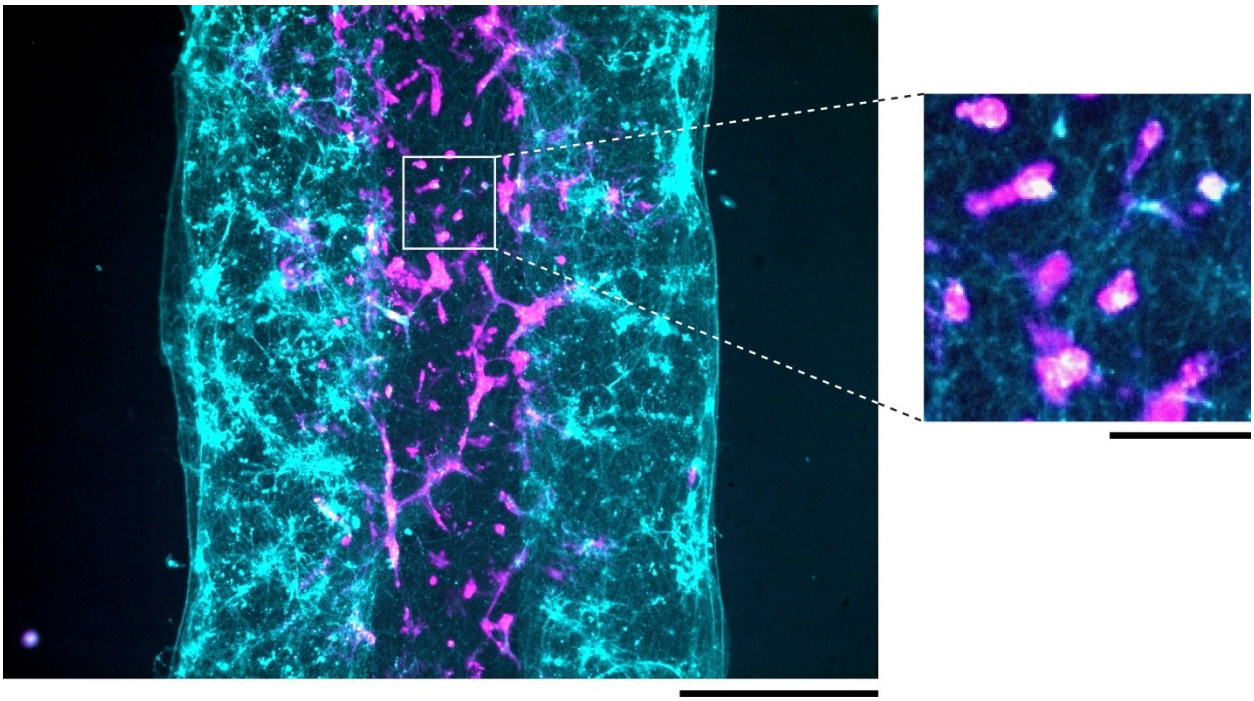


Figure S4. Enlarged image of Fig. 2b (left) and zoom on the middle compartment (right). The middle compartment is occupied by neurites (cyan), shot from neurons in the side compartments, and glial cells (magenta). Moreover, some of glial cells are present in the neuronal compartments, denoting their migration from the central part. Scale bars, 500 µm (left) and 100 µm (right).

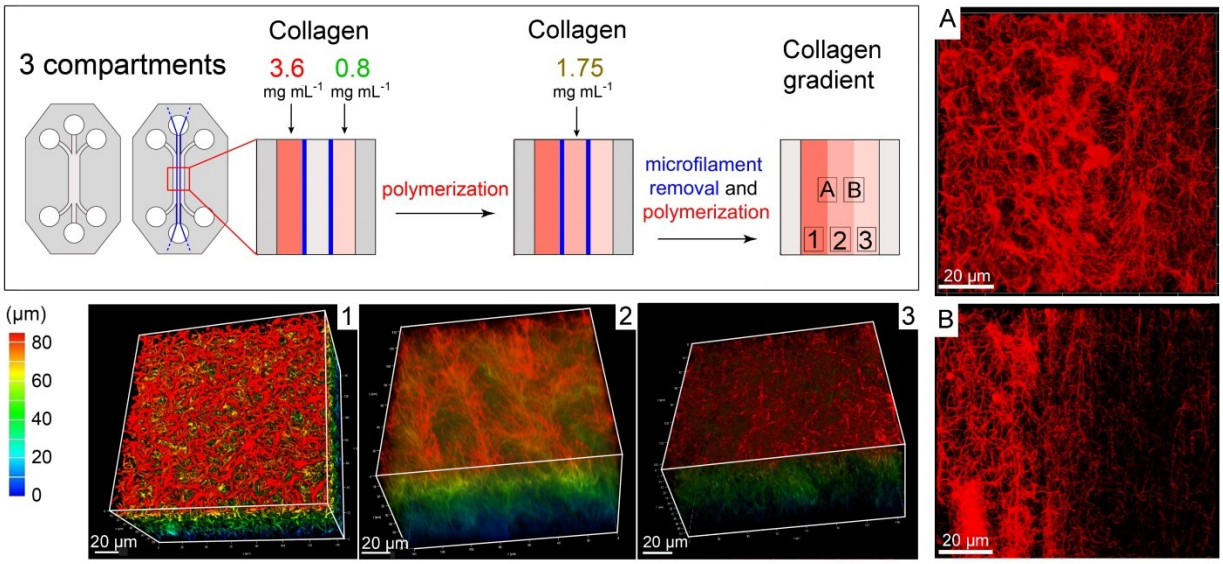


Figure S5. Formation of collagen gel with a stepwise gradient in a microfluidic device. Scheme of the step-by-step process of the collagen gel formation in an 80 µm-high chamber, equipped with 2 removable microfilaments (top left). After polymerizing rat tail collagen at 3.6 and 0.8 mg mL^{-1}, respectively, in the side compartments, collagen solution at 1.75 mg mL^{-1} was introduced in the middle compartment prior to microfilament removal and its polymerization. Detailed protocol is available in ESI Text S1. (1-3) 3D reconstructions of confocal microscopy images of the collagen gel at 3.6, 1.75, and 0.8 mg mL^{-1} in the left, middle, and right part of the chamber, respectively, with colors representing the depth. Collagen fibers are homogeneously distributed in all directions in the chamber. (A, B) Confocal microscopy images at the interface between gels with the highest and the intermediate concentration (A), and the intermediate and the lowest concentration (B). No significant gap was observed.

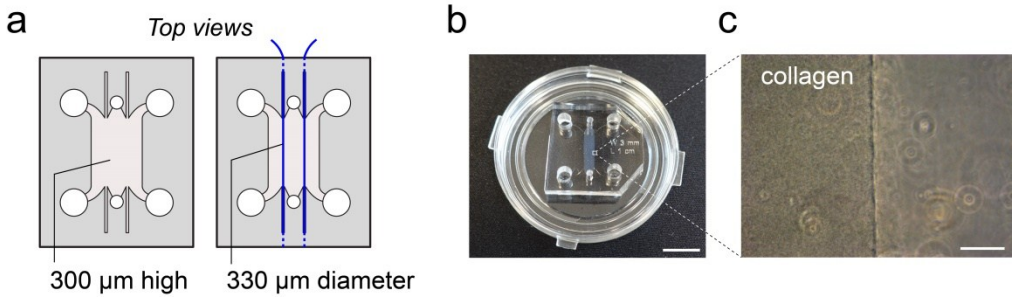
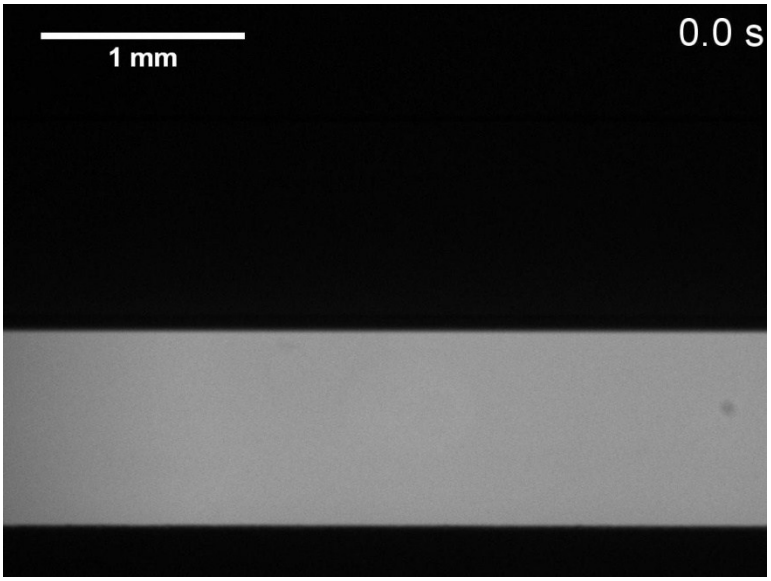


Figure S6. Formation of 300 µm-high bovine collagen I slab in a chamber. (a) Scheme of the device. 330 µm-diameter fishing lines were inserted as removable partitions to a 300 µm-high chamber. The device was bound to a glass-bottom culture dish. Collagen solution (3.4 mg mL^{-1} bovine collagen I and 0.26 % sodium bicarbonate (w/v) in PBS) was introduced in the middle compartment and gelled at 37 °C for 30 min prior to filament removal. Two other compartments were filled with PBS. (b) Photograph of the device after forming the collagen slab. (c) Phase-contrast microscopy image at the edge of the collagen slab. Scale bars, 1 cm (b) and 100 µm (c).



Movie S1. Microfilament removal under fluorescence microscopy observation. A 2 mm-wide chamber was divided into two by a microfilament, with one of the compartment filled with fluorescein solution (bottom) and the other with PBS (top). The filament was removed being pulled from the right. The arrow head in the movie indicates the end of the filament.

Text S1. Formation of collagen gel with a stepwise gradient in a microfluidic device.

All reagents were purchased from Sigma-Aldrich unless otherwise specified.

Collagen gel with a stepwise gradient was formed in a glass-bottom device with 3 transient compartments as follows. Immediately after plasma bonding, the chamber of the device was incubated with 8% (3-aminopropyl)triethoxysilane (APTES) in distilled water (v/v) for 1 h at room temperature, with the solution being renewed every 15 min. Once the residual APTES was washed out by rinsing with water (4-5 times, 100 μL per compartment), 1 % glutaraldehyde in water (w/v) was introduced in the chamber and incubated for 1 h at room temperature with changing solutions every 15 min. Residual glutaraldehyde was then washed out by rinsing with water (4-5 times, 100 μL per compartment). Glutaraldehyde being a cross-linker between APTES bond on the surface and collagen, this pre-treatment of the device helped to stabilize collagen network architectures along the chamber height and to avoid their collapse induced by microfilament removal.

The device was pre-cooled by placing it on ice for at least 10 min before collagen introduction to the device to avoid inhomogeneous collagen polymerization. 100 μL each of collagen solutions was prepared on ice at the concentrations of 0.8, 1.75, and 3.6 mg mL^{-1} separately before introduction to the device, by mixing in the following order: one-tenth volume of 10× PBS (ThermoFisher Scientific), NaOH solution (final concentration to be 10 mM), distilled water, collagen stock solution (Corning), and fluorescently labeled collagen stock solution. 22% (w/w) of collagen was labeled using tetramethylrhodamine (TAMRA) azide (ThermoFisher Scientific). The side compartments of the device were first filled with 20 μL each of the collagen mixtures of the highest and the lowest concentration, respectively, by sucking with a syringe as described in the main text, and the device was placed on ice for 5 min to quench the flow of collagen solutions, followed by incubation at 37 °C under the protection from evaporation for at least 1 h to obtain homogeneous and well positioned gels. After pre-cooling the device again, the middle compartment was filled with the collagen mixture at the intermediate concentration, prior to removal of 2 microfilaments. Then the device was placed on ice for 5 min to quench the collagen flow, and incubated at 37 °C for at least 1 h for collagen polymerization. Collagen gel structures at

different places in the device were visualized by Leica SP8 confocal microscope via TAMRA fluorescence (ESI Fig. S5, 1-3 and A, B).